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Abstract

Cyanobacteria are ubiquitous microorganisms considered as important contributors to the formation of Earth's atmosphere and nitrogen fixation. However, they are also frequently associated with toxic blooms. Indeed, the wide range of hepatotoxins, neurotoxins and dermatotoxins synthesized by these bacteria is a growing environmental and public health concern. This paper provides a state of the art on the occurrence and management of harmful cyanobacterial blooms in surface and drinking water, including economic impacts and research needs. Cyanobacterial blooms usually occur according to a combination of environmental factors e.g., nutrient concentration, water temperature, light intensity, salinity, water movement, stagnation and residence time, as well as several other variables. These environmental variables, in turn, have promoted the evolution and biosynthesis of strain-specific, gene-controlled metabolites (cyanotoxins) that are often harmful to aquatic and terrestrial life, including humans. Cyanotoxins are primarily produced intracellularly during the exponential growth phase. Release of toxins into water can occur during cell death or senescence but can also be due to evolutionary-derived or environmentally-mediated circumstances such as allelopathy or relatively sudden nutrient limitation. Consequently, when cyanobacterial blooms occur in drinking water resources, treatment has to remove both cyanobacteria (avoiding cell lysis and subsequent toxin release) and aqueous cyanotoxins previously released. Cells are usually removed with limited lysis by physical processes such as clarification or membrane filtration. However, aqueous toxins are usually removed by both physical retention, through adsorption on activated carbon or reverse osmosis, and chemical oxidation, through ozonation or chlorination. While the efficient oxidation of the more common cyanotoxins (microcystin, cylindrospermopsin, anatoxin and saxitoxin) has been extensively reported, the chemical and toxicological characterization of their by-products requires further investigation. In addition, future research should also investigate the removal

of poorly considered cyanotoxins (β-methylamino-alanine, lyngbyatoxin or aplysiatoxin) as well as the economic impact of blooms.

Keywords: Cyanobacteria, Ecology, Bloom, Toxin, Drinking Water Treatment, Public Health, Environmental Economy, Microcystin, Anatoxin, Cylindrospermopsin, Saxitoxin, BMAA

1. Introduction

Cyanobacteria were amongst the earliest organisms on Earth and the oxygen released into the atmosphere through their photosynthesis may have been the precursor of the ozone layer (Mur et al., 1999). Presently, while their importance in the evolutionary history of the Earth should not be under-stated, these ubiquitous microorganisms are mostly associated with eutrophic waters. Eutrophication of water resources is often considered as the primary cause of water quality impairment on a world-wide basis. Eutrophication of drinking water resources is primarily caused by excess nutrient loading and storage to lakes and reservoirs due to human activities although climate change will likely play an increasing role in the future (Heisler et al., 2008).

Cyanobacteria are known because of their ability to produce compounds (2-methylisoborneol and geosmin) causing unpleasant tastes and odors in drinking water (Falconer, 1999). However, over the last 2 decades, research priorities have progressively focused on the harmful metabolites also potentially biosynthesised by these microorganisms. Toxins of cyanobacteria (cyanotoxins) include hepatotoxins acting on the liver, neurotoxins acting on the nervous system and dermatotoxins causing skin irritation. Since they have been associated with numerous animal and human poisonings (Briand et al., 2003; Griffiths and Saker, 2003; Kuiper-Goodman et al., 1999; Pouria et al., 1998), cyanotoxins are now a growing environmental and public health concern.

Humans are potentially exposed to cyanotoxins through recreational activities such as bathing in contaminated surface water and through consumption of unsuitably treated drinking water produced from contaminated resources. Therefore, the efficient management and protection of water resources from harmful cyanobacterial blooms is of critical importance to protect human health. For that reason, this extensive review aims to provide a better understanding of cyanobacterial blooms; from the causes of their occurrence and the biosynthesis of toxins to the preventive and remedial options in surface water as well as drinking water supplies. While giving the reader a list of key references for further reading, this review also intends to underline the economic impacts of harmful cyanobacterial blooms and identify major research needs.

2. Occurrence of harmful cyanobacterial blooms

There is no international definition or quantification for what a cyanobacterial bloom is, however, this phenomenon is generally considered as a significant production of biomass over a short period of time correlated with a diminution of phytoplankton diversity. In fact, blooms of cyanobacteria are often mono-specific (or nearly so) and may form a dense layer of cells at the surface of the water visible to the un-aided eye. The formation of cyanobacterial blooms is controlled by environmental factors, and human poisonings are further conditioned by the ability of the individual strains to perform the biosynthesis of cyanotoxins plus subsequent exposure to these harmful metabolites.

2.1. Formation and monitoring of cyanobacterial blooms

Since cyanobacteria are primarily phototrophic microorganisms, groundwater resources are not as vulnerable to bloom formation and associated problems as are surface waters. Cyanobacteria, unlike several types of true algae, usually do not prefer flowing water

although some species have adapted to such conditions. Generally, cyanobacteria flourish in more lentic aquatic ecosystems with relatively high concentrations of primary algal nutrients (nitrogen, phosphorous, and carbon). In some lakes and reservoirs with long residence times, nutrient accumulation and increasing trophic status favor blooms (sometimes nearly mono-specific) of cyanobacteria. As lakes and reservoirs around the world continue to age due to either natural or human causes, the resulting eutrophication will favor cyanobacterial bloom formation over types of true algae. Therefore, the problem of cyanobacterial bloom formation and subsequent risks to human health are an increasingly important and timely topic.

Given the many types of cyanobacteria and the diversity of habitats they have evolved into, predicting all environmental variables required by an individual species to grow and thrive is difficult if not impossible. Generally, the formation of cyanobacterial bloom is regulated by a combination of three primary environmental factors (Fig. 1). The first one is water temperature, several types of cyanobacteria preferring warmer water (25°C or above). Consequently, global warming may increase the frequency and magnitude of cyanobacterial blooms by favoring cyanobacteria among other phytoplankton species (Arheimer et al., 2005; Dale et al., 2006; El-Shehawy et al., 2012; Jöhnk et al., 2008; O'Neil et al., 2012; Paerl and Paul, 2012; Paul, 2008; Wiedner et al., 2007). The second environmental factor influencing cyanobacterial bloom is light exposure. Although several species of cyanobacteria can be considered hetero- or chemo-trophic, most species need a minimum of light availability for photosynthesis to occur. The quality, intensity, and duration of light needed are species-specific. Usually, pigmentation of cyanobacteria protects the cell from photoinhibition due to high light intensities and also improves light harvesting through the absorption across a broader region of the visible spectrum compared to other phytoplankton species (Mur et al., 1999). Therefore, it would appear as if duration of light exposure is a more important growth parameter than light intensity or quality. However, some species are extremely flexible in

their response to light exposure. Indeed, some cyanobacteria can persist in caves for months with virtually no light and are viable and capable of growth immediately following light exposure (Montechiaro and Giordano, 2006). The third factor leading to bloom formation is the trophic status of the aquatic system. Cyanobacterial blooms mainly occur in eutrophic reservoirs (El-Shehawy et al., 2012; Heisler et al., 2008) with N/P ratio ranging from 10 to 15 (Mur et al., 1999). However, another study considering 99 reservoirs indicates that the occurrence of cyanobacterial blooms better correlates with the concentration of N total and P total rather than N/P ratio (Downing et al., 2001).

The presence of cyanobacterial blooms is detected and monitored by different means. A common approach is to measure chlorophyll a (Chl *a*), the primary photosynthetic pigment contained in all phototrophic microorganisms. Chl *a* can be measured either in the field using in situ sensors or samples can be collected for laboratory analyses. However, measurements of Chl a do not discriminate cyanobacteria from algae, which pose a serious limitation on data interpretation. Measuring specific cyanobacterial pigments such as phycocyanin may overcome the problem (Brient et al., 2008; Gregor et al., 2007). In addition, the combined analysis of Chl *a* and phycocyanin provides useful information with respect to the proportion of cyanobacteria among other phytoplankton species.

Another common method of monitoring cyanobacterial blooms is the enumeration and identification of cells by microscope. The advantage of doing so is that cyanobacteria species can be accurately identified and the proportion of cyanobacteria compared to other species in the phytoplankton assemblage. Microscope examination and enumeration has the highest resolution of any other method. Nonetheless, it is time-consuming and requires a relatively high level of taxonomic expertise. Like any other method, microscope identification of a potentially-toxic species of cyanobacteria does not mean it is actively producing toxin. However, enumeration and identification of cyanobacteria by microscope combined with

chemical identification of toxins in the water gives a good indication of the culprit species. In addition to cell counting and identification, new techniques based on polymerase chain reaction (PCR) of genes related to toxin synthesis have been proposed (Al-Tebrineh et al., 2010; Barón-Sola et al., 2012; Baxa et al., 2010; dos Anjos et al., 2006; Hisbergues et al., 2003; Kurmayer and Kutzenberger, 2003; Ostermaier and Kurmayer, 2010).

2.2. Origin of the toxicity

The toxicity of cyanobacteria is related to the biosynthesis of harmful metabolites called cyanotoxins. However, cyanobacterial blooms are not necessarily associated with the occurrence of toxins since not all the strains are toxic (Sarazin et al., 2002). Indeed, cyanotoxins are produced only by the strains having the appropriate genes (Kurmayer and Christiansen, 2009). In addition, even toxic strains do not automatically produce toxins since several of them seem to have the capability to turn certain genes on or off depending upon environmental conditions. Identification of strains having the appropriate genes, however, is as-of-today the best method for determining whether a bloom is or may become toxic. Genes known to have toxin-producing capabilities have been progressively identified in certain strains (Kellmann et al., 2006; Kellmann et al., 2008; Moffitt and Neilan, 2004; Tillett et al., 2000), allowing the development of PCR methods for the specific detection of potentially-toxic cyanobacteria in environmental samples.

Although genetic identification is an excellent tool in determining the biosynthesis of cyanotoxins, strain-specific environmental factors for toxin production exist. The iterative nature of toxin production on a strain-specific level makes generalizations regarding toxin production difficult. Environmental factors include but are not limited to light intensity and exposure time, water movement and flow, allelopathic influences and competition for resources, herbivory and grazing, nutrient concentrations and ratios, water temperature and

salinity, cell division and growth rate (Kosol et al., 2009; Orr and Jones, 1998; Sevilla et al., 2008; Tonk et al., 2005). Indeed, the list of environmental factors and iterations seems almost as large as the list of strains capable of toxin production. Although daunting, much more empirical work needs to be done regarding environmental factors causing toxin production at the individual strain level.

2.3. Literature available

The proliferation of *Nodularia Spumigena* described by Francis (1878) in lake Alexandrina, Australia, is often referred to as the first report of toxic cyanobacterial bloom even though no toxin was identified. Since then, the growing concerns associated with cyanobacteria and their potentially toxic blooms have multiplied the publication of studies on the topic. In fact, the annual amount of publications on cyanobacteria suddenly increased in 1991 and currently keeps growing (Fig.2). In December 2012, the Thomson Reuters Web of Science database retrieved 18642 publications related to cyanobacteria, among which 15350 research articles and 1255 review articles. However, research on cyanobacteria is diverse and articles related to blooms only represent 20% of the publications. In fact the literature available on cyanobacteria covers more than 100 research areas including engineering and the production of biofuels. However, most of the publications remain in the field of water biology (22%), ecological science (18%) and microbiology (16%) while public health and environmental health (0.3%) are sparsely considered.

3. Potential human exposure to cyanotoxins

Cyanotoxins have been associated with numerous animal poisonings worldwide, but they are also a threat for human health. As presented in Fig. 1, human exposure to these harmful metabolites can have 3 major origins. One route of exposure is the ingestion of

cyanobacteria-based food ingredients or shellfish which previously bioaccumulated toxins through filtration of contaminated water (Ibelings and Chorus, 2007; Johnson et al., 2008; Rellán et al., 2009; Saker et al., 2005). Another exposure route is possible through dermal contact and accidental inhalation/ingestion during recreational activities in waters subjected to a toxic bloom. The third route of exposure could be caused by the ingestion of drinking water produced from a contaminated resource (Byth, 1980; Griffiths and Saker, 2003). Depending upon the population served and type of treatment prior to delivery, this third exposure route could affect a relatively large number of people. Some examples of intoxication and relevant guidelines are provided with the chemical and biological properties of cyanotoxins.

4. Occurrence and properties of cyanotoxins

The word cyanotoxin refers to more than a hundred compounds that may strongly differ in their chemical structure and toxicological property (Table 1). They are usually arranged into 3 classes according to their target organ: hepatotoxins that induce liver injuries, neurotoxins that alter the neuromuscular transmission and dermatotoxins that induce skin irritation.

4.1. Cyanobacterial hepatotoxins

4.1.1. Microcystins

Microcystins (MCs) form the main family of cyanotoxins since they are the most frequently studied and the most widespread. For example, their occurrence has been reported in Asia, Europe, North Africa, North America and Scandinavian countries (Fristachi and Sinclair, 2008). MCs were named according to *Microcystis*, the first genera of cyanobacteria associated with their biosynthesis. However, MCs are also produced by *Oscillatoria*, *Nostoc*,

Anabaena and *Anabaenopsis* (Kaebernick and Neilan, 2001). As mentioned previously, toxin synthesis is a complex process influenced by environmental conditions and depending on the genetic properties of each cyanobacterial strain. In the last decade, the gene cluster *mcyA-J* was identified as the origin of MCs biosynthesis. In fact, *mcyA-J* codes for a multienzyme complex including peptide synthetase and poliketide synthase, allowing the components of the toxin to be assembled non-ribosomally (Dittmann and Wiegand, 2006; Kaebernick and Neilan, 2001).

As presented in Fig. 3, MCs are cyclic compounds enclosing 7 amino acids. Among them, the unusual Adda amino acid is often associated with the toxicity of the molecule because of its conjugated diene (Dawson, 1998). In addition, X and Z are usually referred to as variable amino acids which multiple combinations make the difference between more than 70 variants of the toxin (Sivonen and Jones, 1999). Then, each variant is identified by the initials of X and Z. For example, the common MC which has leucine (initial L) and arginine (initial R) should be identified as MC-LR.

MCs are water-soluble and stable molecules (Sivonen and Jones, 1999). Once absorbed by the organism, they are quickly concentrated in the liver (Fischer et al., 2000) and bind to the protein phosphatase (Dawson, 1998; Gupta et al., 2003; Kuiper-Goodman et al., 1999; MacKintosh et al., 1990). Depending upon dose and body weight, the inhibition of protein phosphatase may lead successively to the accumulation of phosphorylated proteins in the liver, cell necrosis, massive haemorrhage and death. For example, the lethal dose 50 (LD_{50}) of MC-LR after intraperitoneal (i.p.) injection in mice ranges from 25 to 150 µg/kg (Kuiper-Goodman et al., 1999). This value may differ according to the MC variant but MC-LR is usually used as a reference. MCs are also considered to be potential tumor promoters (Falconer, 1991; Nishiwaki-Matsushima et al., 1992).

Numerous animal and human intoxications by MCs have been reported (Hilborn et al., 2007; Soares et al., 2006; Stewart et al., 2008). Most of the human poisonings were limited to gastro-enteritis (Kuiper-Goodman et al., 1999; Teixeira et al., 1993) but, when water containing the toxin was used for hemodialysis, MCs also caused the death of 60 patients at the Brazilian dialysis centre of Caruaru in 1996 (Azevedo et al., 2002; Jochimsen et al., 1998; Pouria et al., 1998; Yuan et al., 2006). Consequently, the World Health Organization considered the MC-LR no observable adverse effect level (NOAEL) of 40 µg/kg/d obtained after 13 weeks mice oral exposure (Fawell et al., 1999) and derived a guideline of 1 µg/L as a maximum value for MC-LR in drinking water (WHO, 1998).

4.1.2. Nodularins

Nodularins (NODs) have been reported mainly in Australia, New Zealand and the Baltic Sea (Sivonen and Jones, 1999; van Apeldoorn et al., 2007). Associated only with *Nodularia spumigena* (Kaebernick and Neilan, 2001), their biosynthesis is regulated by genes and performed non-ribosomally according to a mechanism similar to that involved in MC production (Dittmann and Wiegand, 2006).

As shown in Fig. 3, NODs are cyclic pentapeptides structurally similar to MCs, including the Adda moiety but only one variable amino acid Z. So far, 9 variants of this water soluble and stable toxin have been identified (Codd et al., 2005), the most common being NOD-R with Arginine as variable amino acid.

Like MCs, NODs are hepatotoxins acting through the inhibition of protein phosphatase and are potential tumor promoters. According to the variant, the LD_{50} of NODs in mice after i.p. injection ranges from 30 to 70 µg/kg (van Apeldoorn et al., 2007). However, no human intoxication with NODs has been reported so far, and no guidelines have been proposed for drinking water due to the lack of suitable toxicological data.

4.1.3. Cylindrospermopsin

Cylindrospermopsin (CYL) has been initially detected in Australia (Griffiths and Saker, 2003; Saker et al., 1999), New Zealand (Stirling and Quilliam, 2001), and Thailand (Li et al., 2001). Consequently, CYL was considered as a tropical toxin until its recent characterization in temperate areas including Germany (Fastner et al., 2003; Fastner et al., 2007; Rücker et al., 2007) and France (Brient et al., 2009). CYL was named according to *Cylindrospermopsis raciborskii*, but other cyanobacteria like *Aphanizomenon ovalisporum*, *Raphidiopsis curvata* and *Umezakia natans* can also perform the biosynthesis of the toxin (Banker et al., 1997; Fristachi and Sinclair, 2008). Similarly to MCs, the synthesis of CYL seems to be regulated by genes coding for polyketide synthase and peptide synthetase that gather toxin's components non-ribosomally (Schembri et al., 2001).

As shown in Fig. 3, CYL is a 415 Da tricyclic alkaloid enclosing a guanidine entity along with a uracil moiety potentially responsible for the toxicity (Banker et al., 2001). So far, the alteration of the hydroxyl group near the uracil moiety leads to the identification of 2 other variants: 7-epicylindrospermopsin with a different OH orientation (Banker et al., 2000) and the non-toxic deoxycylindrospermopsin without OH (Li et al., 2001; Norris et al., 1999).

CYL is highly water-soluble with a half-life greater than 10 days in high purity water (Chiswell et al., 1999). After ingestion, the toxin mainly impacts the liver via the irreversible inhibition of protein synthesis leading to cell death (Froscio et al., 2003; Froscio et al., 2008; Metcalf et al., 2004). For example, CYL exhibits a 2100 µg/kg LD_{50} in mice, 24 hour after i.p. injection (van Apeldoorn et al., 2007). However, CYL exposure can also lead to fetal toxicity (Rogers et al., 2007), tumor initiation (Falconer and Humpage, 2001), micronucleus induction and chromosome loss (Humpage et al., 2000).

The most famous case of human intoxication by CYL occurred in 1979 in Australia and is often referred to as the Palm Island mystery disease (Bourke et al., 1983; Byth, 1980; Griffiths and Saker, 2003). The application of an algaecide to eliminate a bloom of cyanobacteria in the water supply resulted in CYL release and over 100 admissions of children to the local hospital for gastroenteritis associated with the consumption of contaminated drinking water. Therefore, based on the 30 µg/kg/d NOAEL observed on mice orally exposed to CYL during 11 weeks, 1 µg/L was proposed as a guideline for maximum concentration in drinking water (Humpage and Falconer, 2003).

4.2. Cyanobacterial neurotoxins

4.2.1. Anatoxin-a

The occurrence of anatoxin-a (ANTX-a) was reported in USA (Osswald et al., 2007), Africa (Ballot et al., 2003; Krienitz et al., 2003), Asia (Namikoshi et al., 2003; Park et al., 1993; Park et al., 1998) and Europe (Carrasco et al., 2007; Gugger et al., 2005; Viaggiu et al., 2004). This toxin is mainly associated with 3 genera of cyanobacteria: *Anabaena*, *Aphanizomenon* and *Planktothrix* (Osswald et al., 2007; van Apeldoorn et al., 2007). The biosynthesis of ANTX-a has not been completely described yet, but the responsible genes have been identified (Cadel-Six et al., 2009; Mejean et al., 2010).

As presented in Fig. 4, ANTX-a is a 165 Da alkaloid with a variant called homoanatoxin-a resulting from the methylation of the carbon at the extremity of the ketone function (van Apeldoorn et al., 2007). ANTX-a is highly water-soluble but unstable at pH above 10 and transformed into a non-toxic form by sunlight exposure.

Once in the organism, ANTX-a induces paralysis by fixation on acetylcholine receptors without being degraded by acetylcholinesterase (Osswald et al., 2007). Consequently, death can occur by respiratory arrest when muscles involved in breathing

activity are affected. For example, the LD_{50} 24 hours after i.p. injection in mice is 375 µg/kg (van Apeldoorn et al., 2007).

ANTX-a has been responsible for various animal poisonings resulting in vomiting, convulsion and respiratory arrest (Gugger et al., 2005; Henriksen et al., 1997; Krienitz et al., 2003; Wood et al., 2007), but no human poisonings have yet been reported. So far, there is no official guideline for ANTX-a in drinking water because of dissimilar results in subacute toxicity studies (Kuiper-Goodman et al., 1999), but 3 µg/L has been suggested (van Apeldoorn et al., 2007).

4.2.2. Anatoxin-a(s)

Anatoxin-a(s), known as ANTX-a(s), has been identified in restricted areas including the United States, Scotland, Denmark and Brazil (Molica et al., 2005; Onodera et al., 1997; Sivonen and Jones, 1999). The toxin has been associated only with *Anabaena* strains, but the biosynthesis has not been completely explained yet.

As shown in Fig. 4, ANTX-a(s) is a 252 Da phosphate ester of a cyclic N-hydroxyguanine (Sivonen and Jones, 1999; van Apeldoorn et al., 2007). Once absorbed in the organism, ANTX-a(s) inhibits acetylcholinesterase (Molica et al., 2005) and induces muscular paralysis with potential death by respiratory arrest. Very few toxicological studies have been carried out and only the LD_{50} by i.p. injection into mice is available: 20-31 µg/kg (van Apeldoorn et al., 2007). Consequently, no guideline has been proposed yet for ANTX-a(s) in drinking water.

4.2.3. Saxitoxins

In freshwaters, saxitoxins (STXs) have been detected in Australia and USA (Kuiper-Goodman et al., 1999). While *Anabaena circinalis* and *Aphanizomenon flos-aquae* seems to

be the main associated cyanobacteria, *Lyngbya wollei* and *Cylindrospermopsis raciborskii* were also shown to perform the biosynthesis of the toxin (Nicholson et al., 2003). In sea water, STXs are also produced by some dinoflagellates. Again, with the identification of a relevant gene cluster, the knowledge of STXs biosynthesis has been improved (Kalaitzis et al., 2010).

As presented in Fig. 4, STXs are tricyclic compounds ranging from 241 to 491 Da that can be non-sulphated, singly sulphated or doubly sulphated (Nicholson et al., 2003; van Apeldoorn et al., 2007). These water-soluble toxins can persist over 90 days in freshwater (Jones and Negri, 1997), but they are altered by high temperatures and degraded into more toxic variants (Sivonen and Jones, 1999).

STXs, also known as paralytic shellfish poisons, block sodium ion channels in nerve axon membrane and induce nerve dysfunction, paralysis then death due to respiratory failure (van Apeldoorn et al., 2007). For example, the LD_{50} of the most toxic variant in mice was shown to be 10 µg/kg after i.p. injection.

Over the last century, STXs have been associated with numerous human intoxications resulting in numbness, complete paralysis and even death (Kuiper-Goodman et al., 1999). However, no intoxication through drinking water has been documented so far. While no official guideline has been proposed for STXs in drinking water, Australia is considering a 3 µg STX eq/L to be used (van Apeldoorn et al., 2007).

4.2.4. β-N-methylamino-L-alanine

The cyanotoxin β-N-methylamino-L-alanine (BMAA) has been recently identified in England (Metcalf et al., 2008), Peru (Johnson et al., 2008), South Africa (Esterhuizen and Downing, 2008), China (Li et al., 2010) and Florida (Brand et al., 2010). BMAA has not been extensively studied yet but a recent work indicates that this toxin may be produced by all

known groups of cyanobacteria (Cox et al., 2005). Indeed, cyanobacteria possess genes coding for cysteine synthase-like enzyme and methyl transferase, both being involved in BMAA biosynthesis (Aráoz et al., 2010a).

BMAA is a 118 Da non-protein amino acid shown in Fig. 4. It acts mostly on motor neurons by fixation on glutamate receptors. In addition, BMAA could also cause intraneuronal protein misfolding, the characteristic of neurodegeneration (Banack et al., 2010a). In fact, there are assumptions that BMAA could be associated with various neurodegenerative diseases such as the amyotrophic lateral sclerosis/parkinsonism dementia complex in Guam or Alzheimer's disease (Banack et al., 2010a; Murch et al., 2004; Pablo et al., 2009). However, due to the lack of toxicological data such as LD_{50} or NOAEL, no guideline has been proposed for BMAA in drinking water.

4.2.5. Other neurotoxins

In addition to the common freshwater toxins presented previously, a recent review also mentioned the existence of 3 other marine cyanobacterial neurotoxins (Aráoz et al., 2010a). Antillatoxin, kalkitoxin and jamaicamide are lipopeptides produced by *Lyngbya majuscula* that induce neurotoxicity through the interaction with voltage-gated sodium channels. However, due to the limited data available for these compounds, their chemical and biological properties won't be further developed in this paper.

4.3. Cyanobacterial dermatotoxins

Cyanobacterial dermatotoxins include aplysiatoxins (APTXs) and lynbyatoxins (LTXs) mainly produced by *Lyngbya majuscula* (van Apeldoorn et al., 2007). So far, these toxins have been detected only in marine water. After human exposure, characteristic symptoms of poisoning include dermatitis as well as oral and gastrointestinal inflammation

resulting in diarrhea (Nagai et al., 1996; Osborne et al., 2007). In addition, APTXs and LTXs are also potent tumor promoters through the activation of protein kinase C (van Apeldoorn et al., 2007). However, due to the lack of data, their chemical and biological properties won't be further developed in this paper.

4.4. Literature available

MCs are probably the most common and the most well known toxic metabolites of cyanobacteria but STX and ANTX were the first cyanotoxins to be studied in articles from the early 1960s. Indeed, other toxins were only considered in studies published since the 1980s.

The annual amount of publications on cyanotoxins dramatically increased over the last two decades (Fig. 2), mainly because of the constant progress in analytical science and the growing awareness of the public health risk associated with these cell metabolites. In December 2012, the Thomson Reuters Web of Science database retrieved 5293 publications related to cyanotoxins, among which 4366 research articles, 467 proceedings and 198 review articles. However, these publications are largely unevenly distributed among the different toxins, as shown in Fig. 2. Indeed, with 2971 publications MCs account for more than 56% of the overall literature. STXs come in second position with 27% of the literature but most of these articles are actually associated to red tides rather than cyanobacteria. While NODs, ANTX and CYL individually represent less than 10% of the literature available on cyanotoxins, Fig. 2 reveals BMAA, LTX and APTX are largely understudied with less than 2% of the total amount of publications.

The literature available on cyanotoxins is also unevenly distributed between more than 100 research areas. Indeed, most of the studies focus on toxicology (24%), ecology (19%), chemistry (18%) and pharmacology (17%). While water biology, biochemistry and molecular biology still account for 13% of the literature, each of engineering and water resources only

represent 7%. With less than 5% of the overall amount of publications, other research areas including economy, public health or epidemiology are even less considered.

5. Detection and quantification of cyanotoxins

A wide range of methods are available for the analysis of cyanotoxins (Table 2; Fig. 5). These include numerous techniques relying on biological and physico-chemical approaches. However, according to the method employed and the kind of results expected, samples often require specific preparation before analysis.

5.1. Sample preparation

After field sampling, samples should be stored at low temperature (4°C) and analyzed as soon as possible in order to prevent any alteration of toxin distribution (intracellular/extracellular). Sample preparation differs according to the kind of toxin analyzed. As shown in Fig. 5, direct filtration of the sample only allows the detection of extracellular toxins but an additional step inducing the lysis of cyanobacteria retained on the filter would allow separate detection of intracellular toxins. However, cell lysis prior to filtration allows the simultaneous detection of both extracellular and intracellular toxins without determining their repartition. Cell lysis is often obtained by freezing-thawing cyanobacteria or adding methanol in the sample (or onto the filter). Both methods directly damage cell membranes and release intracellular toxins (Harada et al., 1999).

In addition, cyanotoxins in the filtrate may also undergo further purification and concentration, usually through solid phase extraction (SPE). In this case at least 500 mL of filtrate are poured through a cartridge containing a sorbent, usually a reversed phase C18. Then toxins are eluted using methanol and water (usually 90% methanol). In order to further increase the concentration of toxins, the cartridge eluate may be partially evaporated. This

practice can concentrate cyanotoxins by 3 orders of magnitude, which consequently improves the detection limit of any subsequent analytical method.

5.2. Biological approach for the analysis of cyanotoxin

Cyanotoxins can be detected and quantified through a biological approach relying on *in vivo* assays, immunological assays or biochemical assays, each with specific benefits and limitations.

5.2.1. Toxin analysis by *in vivo* assays

Mouse bioassay is likely the most well known *in vivo* assay. In fact, it was the first method developed to detect cyanotoxins in water even if it was actually designed to assess their biological effects. The procedure consists in the i.p. injection of the sample in a minimum of 3 mice followed by their necropsy after 24 hours (Falconer, 1993). The observation of different symptoms reveals the presence of hepatotoxins or neurotoxins in the matrix. For instance, while increasing the weight and the volume of the liver, the hepatotoxins also induce the alteration of hepatic cells (Falconer, 1993). However, mouse bioassay does not allow the exact identification of the toxin (MCs, NODs…) in the sample.

Despite its low sensitivity, mouse bioassay can be considered as a semi-quantitative method when comparing the extent of the lesions to those observed on mice exposed to different concentrations of a standard toxin. In this case, the results are often expressed as equivalent of the standard toxin (usually MC-LR). Due to ethical issues with respect to animal experiments and the development of new methods (faster, more sensitive and more specific) for cyanotoxin measurement, the use of mouse bioassay is mostly limited to toxicological research.

Alternative *in vivo* bioassays relying on less controversial organisms, like crustacean larvae, have also been developed in order to quantify cyanotoxins (Kaushik and Balasubramanian, 2012). Larvae of the selected organism (for example *Artemia*, *Daphnia* or *Thamnocephalus*) are exposed to toxins through the incubation in a growth medium diluted in a specific volume of the sample to be tested. However, while these assays can be performed in a 96-well plate, they are still not specific and have a strong potential for interferences due to matrix effects.

5.2.2. Toxin analysis by immunological assays

Cyanotoxins can also be detected through recognition and binding to specific antibodies. For example, various ELISA (Enzyme-Linked ImmunoSorbent Assay) kits are commercially available for the detection of MCs in water (Carmichael and An, 1999; Hilborn et al., 2005; Lindner et al., 2004; Rapala et al., 2002). According to the antibody and the procedure employed, these extremely sensitive methods can achieve a detection limit as low as 4 ng/L with an upper quantitation limit (due to saturation) close to 2 µg/L for MC-LR (Lindner et al., 2004). Therefore, while ELISA is successfully employed for the detection of MCs, specific antibodies have also been designed to apply this method to the detection of CYL and STXs (Bláhová et al., 2009; Campbell et al., 2009)

However, detection methods based on ELISA also have some limitations. For instance, the different MCs variant cannot be distinguished and the results have to be expressed as equivalent MC-LR/L. In addition, cross reactivity (even limited) with other compounds in the sample may lead to overestimate the concentration of the toxins.

5.2.3. Toxin analysis by biochemical assays

Since MCs and NODs are potent inhibitors of protein phosphatase, these toxins can be detected using a protein phosphatase inhibition assay known as PPIA (Almeida et al., 2006; Bouaïcha et al., 2002; Heresztyn and Nicholson, 2001; Ortea et al., 2004; Rapala et al., 2002). Before incubation with the relevant substrate, the enzyme is exposed to an aliquot of the sample containing the toxin. Measuring the absorbance of the mixture at a specific wavelength allows the detection of the substrate (or its transformation product) and the assessment of the enzyme activity, which is inversely proportional to the concentration of the toxin.

According to the method employed, PPIA can ensure toxin detection within a few hours for a large number of samples. Such procedure allows the quantification of MC-LR with a detection limit reaching 0.01 µg/L (Almeida et al., 2006). However, PPIA cannot distinguish co-occurring variants of MCs and cannot distinguish MCs from NODs. Therefore, results are often expressed as equivalent MC-LR/L. In addition, when analyzing bloom-containing water, interferences with unknown compounds leading to overestimation or underestimation of toxin concentration should be considered. Moreover, since PPIA detects only MCs and NODs, further analysis should be undertaken to detect other cyanotoxins potentially occurring in the sample.

5.3. Physico-chemical approach for the analysis of cyanotoxins

Cyanotoxins can also be analyzed through a physico-chemical approach often relying on two steps, the separation of compounds present in the sample by chromatography followed by their quantification with specific detectors. Depending on compatibility, a chromatographic technique can be coupled to different detection technique and reciprocally.

5.3.1. Separation techniques

Separation techniques are commonly employed since they allow the discrimination of several co-occurring toxins within a single analysis, even with a non-specific detector. Indeed, toxins are then identified when pairing the separation profile of the sample with references obtained from the analysis of standards or purified compounds in the same conditions.

Liquid chromatography (LC), usually with a reversed phase C18 or a HILIC column and methanol/water or water/acetonitrile as a mobile phase, is likely the most common separation method for cyanotoxins since it allows flexibility, rapidity and adaptability to a wide range of detector relying on UV absorbance, fluorescence or mass spectrometry. Gas chromatography (GC) is also used as a separation method for cyanotoxins (Kaushik and Balasubramanian, 2012) but to a lower extent. Indeed, some cyanotoxins like MCs are large molecules and not really volatile and GC separation requires more complex sample preparation that may include derivatization. Capillary electrophoresis (CE) performing separation of compounds according to their mass and charge can also be employed in the analysis of cyanotoxins like ANTX, CYL and MCs (Vasas et al., 2004). However, even though detection by mass spectrometry or fluorescence (after derivatization) could provide sensitivity, a recent study indicates CE is not considered sufficiently robust yet to be used for routine analysis (Kaushik and Balasubramanian, 2012).

5.3.2. Detection by UV absorbance and fluorescence

Monitoring UV absorbance was one of the first techniques to detect cyanotoxins after LC separation. Indeed, MCs or CYL have specific UV spectra with a maximum absorbance at 240 nm and 262 nm respectively (Merel et al., 2009; Merel et al., 2010b). However, detection by UV absorbance offers only limited sensitivity and low specificity. For instance, MCs have similar UV spectra (Harada et al., 1999) and the identification of the variant depends only on

the retention time. Consequently, only 7 variants with analytical standards available can be reliably identified and quantified by LC-UV. Those remaining have to be quantified and reported as MC-LR equivalent. In addition, the lack of specificity further increases when monitoring only the absorbance at selected wavelengths instead of the full UV spectrum and potential interferences should be anticipated in complex matrices like bloom-containing water.

Detection by fluorescence is also commonly used after LC separation. Therefore, methods based on this technique have also been developed for the detection of cyanotoxins as an alternative to UV absorbance (Harada et al., 1997; Kaushik and Balasubramanian, 2012). Indeed, fluorescence detection usually significantly improves sensitivity. However, cyanotoxins do not naturally fluoresce implying the addition of a derivatization process during the sample preparation.

5.3.3. Detection by mass spectrometry

Mass spectrometry (MS) became increasingly common over the last decades due to its high sensitivity in comparison to other detection methods and its availability for both LC and GC. In addition, MS detects compounds based on their mass and charge, which therefore limits the potential for interferences and improves selectivity. Moreover, the development of tandem mass spectrometry (MS/MS) also enhanced specificity by further discriminating compounds with similar mass and charge through their specific fragmentation pattern when colliding with molecules of inert gas.

GC-MS methods have been developed and proven successful for the analysis of some cyanotoxins like MCs but very complex procedures are required for sample preparation. Indeed, published GC-MS methods for the analysis of MCs even require sample oxidation, post-treatment to remove remaining reagents and derivatization. Consequently, cyanotoxins

are mostly detected by LC-MS or LC-MS/MS, allowing simultaneous detection of a larger amount of toxins with a simpler sample preparation procedure (Kaushik and Balasubramanian, 2012). Specific details about analytical conditions and methods can be found in the relevant references provided in Table 2.

Cyanotoxins can also be detected by MS without preliminary chromatographic separation, particularly with time of flight (TOF) mass spectrometers. For example MALDI-TOF instruments can be used to perform toxin analysis in very small sample volume such as cell colonies (Kaushik and Balasubramanian, 2012). Molecules enclosed in the dried and solid sample are ionized by a laser beam and accurately identified through the high mass resolution provided by the TOF instrument. However TOF mass spectrometers usually tend to be less sensitive than other mass spectrometers from the same generation.

5.4. Challenges for the analysis of cyanotoxins

Cyanotoxins enclose a wide range of compounds with different properties and, despite the major progress in analytical science over the last decades, their analysis still remains challenging. The first challenge consists in developing new methods able to identify and quantify simultaneously as many toxins as possible along with their different variants. Indeed, there is no single method yet able to detect all the cyanotoxins potentially occurring in a water sample. For instance, while biological methods are usually toxin specific, physical methods like LC-MS also have limitations and quantifying several cyanotoxins might require several sample analysis using different chromatographic conditions. The second challenge consists in continuously improving method robustness and detection limit. For example, sample preparation may result in a partial loss of analytes, biological methods are subject to matrix interference called cross reaction and physical methods like LC-MS suffer matrix effect called ion suppression. While cross reaction cannot be readily accounted for, spiking the sample a

stable isotope of the analyte can correct for eventual loss during sample preparation and ion suppression. However, stable isotopes of cyanotoxins are not commercially available yet, meaning that without such correction, concentration reported by LC-MS in bloom-containing water might have been partially underestimated. Finally, another challenge consists in making the analysis of cyanotoxins faster, cheaper and feasible *in situ*. For example sample preparation is increasingly automated but also more commonly performed online, which lower the volume of sample necessary and increase throughput. However, the current state of the art does not yet allow sensitive mass spectrometric analysis *in-situ*, but serious progress have been made on this topic with biological detection methods. Indeed, immunological method have been adapted to develop strips for measurement of toxins in the field (Humpage et al., 2012) but further research is still needed to achieve the same quantification capabilities than laboratory based procedures.

6. Management of cyanobacterial blooms in surface water

The management of cyanobacterial blooms in surface water is a complex task which aims to prevent, monitor, and treat such phenomenon while circumventing human health issues. Different strategies can be applied to reach these objectives, but each potentially contaminated site has to be carefully considered in order to select the more suitable approach.

6.1. Prevention of bloom occurrence

The concentration of nutrients (primarily species of C, N, and P) for cyanobacterial and algal growth in water is a key factor in promoting bloom formation and overall eutrophication of surface waters across the world. Decreasing the inputs of nutrients in surface water is a primary, but long term strategy to prevent the occurrence of cyanobacterial blooms (Downing et al., 2001; Paerl et al., 2011a; Paerl et al., 2011b). First effort to reduce

nutrient inputs was made in 1970s by increasing and improving wastewater treatment instead of rejecting untreated effluents directly into surface waters. Within a few years, this approach significantly decreased bloom occurrence in numerous surface waters in the US such as Lake Washington, Lake Erie and the Potomac River (Heisler et al., 2008). Now, as most of the largest cities in developed countries operate wastewater treatment facilities, further reduction of nutrient inputs can be achieved by improving agricultural practices with particular emphasis on the use of fertilizers and manure spreading (Chorus and Mur, 1999). However, acting on such diffuse, non-point sources of nutrients is a long term strategy. In addition, results may not be observed immediately since the high amount of nutrients already in lake sediments and released through physico-chemical processes (autochthony) may still favor the growth of cyanobacteria and overall eutrophication years into the future.

The management and control of cyanobacterial blooms is really the control and management of nutrients from two sources, from outside of the lake or reservoir (allochthonous) and from within the reservoir (autochthonous). Controlling non-point sources of nutrients is difficult. Indeed some nutrients (i.e. nitrogen) are deposited atmospherically in lakes and reservoirs from remote sources thousands of kilometers away. Also, almost every human activity in the watershed affects nutrient loading to lakes and reservoirs. For instance, urbanization in general is a huge source of nutrient delivery along with climate change and wildland fires also resulting from anthropogenic activities.

To control non-point sources of pollution, the US Environmental Protection Agency (EPA), under Section 303(d) of the Clean Water Act, has implemented a Total Maximum Daily Load Program (TMDL) for surface waters of the US (US EPA 2012a). The TMDL program is run on a state-by-state basis and attempts to:

1. Identify Quality Limited Waters. States must identify and prepare a list (US EPA 2012b) of waters that do not or are not expected to meet water quality standards after applying existing required controls (e.g. minimum sewage treatment technology).
2. Establish Priority Waters/Watersheds. States must prioritize waters/watersheds and target high priority waters/watersheds for TMDL development.
3. Develop TMDLs- For listed waters, States must develop TMDLs that will achieve water quality standards, allowing for seasonal variations and an appropriate margin of safety. A TMDL is a quantitative assessment of water quality problems, contributing sources, and load reductions or control actions needed to restore and protect individual waterbodies.

The TMDL process attempts to control and reduce non-point sources of pollution while taking into account the complexity involved with many different types of aquatic ecosystems. It can be a lengthy process but it is relatively comprehensive in scope.

Although the TMDL process is a key tool for US regulation and control of non-point sources of pollution delivered to surface waters of the US (allochthonous inputs), it is less robust at controlling nutrient recycling (autochthonous) within lakes and reservoirs. To address the problem of nutrient recycling and a reduction in cyanobacterial biomass requires highly specialized techniques and expertise. Also, due to large differences in inherent variability within aquatic systems, the "one-size-fits-all" approach almost never works. The selection of what specific technique or set of techniques works the best in any given lake or reservoir is site-specific. Lake and reservoir management should be on-going so that data can be collected and analyzed in light of changing environmental conditions. Phytoplankton in lakes and reservoirs is a highly dynamic assemblage of organisms and therefore, the management of these aquatic resources should be equally as dynamic.

It is beyond the scope of this paper to address all the limnological concepts behind lake and reservoir management for a reduction in trophic status and, therefore, in bloom

formation. The most important tool in reducing bloom formation is an ecological understanding of what species are present and their requirements for growth. This knowledge needs to be first and foremost otherwise, all management techniques aiming to reduce the prevalence and magnitude of cyanobacterial blooms are hit-or-miss. Just a few lake and reservoir management techniques known to control nutrient recycling are provided below:

- Aeration and mixing (including hypolimnetic aeration)
- Sediment dredging
- Sediment inactivation (usually by using aluminium sulfate)
- Dosing with aluminium sulfate to bind P and make it limiting for cyanobacterial growth.
- Algaecides to reduce cyanobacteria and algal biomass (and decrease bottom deposits).

Techniques should be carefully chosen and expert limnologists and lake managers consulted. Results need to be data-driven rather than anecdotal and on-going management as well as data collection should be implemented. Unfortunately, there are no quick fixes for reducing trophic status and bloom formation so goals and objectives might take some time to be achieved.

6.2. Eradication of occurring cyanobacterial blooms

The usual technique to eradicate a bloom of cyanobacteria consists in applying an algaecide, usually copper sulfate (Hrudey et al., 1999). Indeed, cyanobacteria are among the microorganisms most vulnerable to Cu^{2+} which affects electron transport in the photosystem and the activity of fundamental enzymes (Hrudey et al., 1999; Le Jeune et al., 2006).

While algaecides efficiently eliminate occurring blooms, they also induce cell lysis and subsequent release of intracellular toxins (Jones and Orr, 1994; Peterson et al., 1995). For example, microscopic observation of *Microcystis aeruginosa* exposed to copper sulfate (650

µ/L for 24 hours) indicates massive cell membrane alteration correlated with an increasing concentration of extracellular MC-LR (Kenefick et al., 1993). In addition, Cu^{2+} tends to precipitate and accumulate in sediments, causing copper toxicity issue and allowing cyanobacteria to bloom again after a few weeks.

Even though a recent study addresses the issue of copper toxicity by the application of hydrogen peroxide instead of copper sulfate (Matthijs et al., 2012), algaecides should be avoided has much as possible since it is barely a short term solution to blooms which further leads to both ecological and public health risk through copper accumulation in sediment and potentially significant toxin release (Griffiths and Saker, 2003). Thus, once a bloom occurs in surface water there is no ideal curative measure, which strengthen the importance of preventive measures described previously.

6.3. Bloom monitoring and prevention of health issues

Several countries besides the US, such as Germany, Finland, France or the Netherland, have developed specialized monitoring procedures (Chorus, 2005; Ibelings, 2005; Rapala et al., 2005). Differences in procedures exist yet but they all share some commonality. As previously mentioned, phytoplankton counts and identification should be on-going and must take into account seasonal variability in assemblage changes. Based upon total biomass, if cyanobacteria are predominant, then toxins should be analyzed. If toxin concentrations are low (different ranges of high, intermediate, and low by country) there may be no need for any action other than public warning and awareness. On the contrary, intermediate toxin concentration leads to public information with restriction of recreational activities while high toxin concentration leads to prohibition of recreational activities, including fishing. Once toxins are detected in surface water, their concentration has to be monitored until they become non-detected again (biodegradation, etc.).

As it is impossible to constantly supervise every surface waters for toxin activity, public information should be the primary means to prevent human intoxication.

7. Drinking water treatment and cyanobacteria

Until preventive measures (unique but long term solutions to blooms) discussed previously successfully prevent the occurrence of cyanobacterial blooms in drinking water resources, drinking water treatment should be able to protect consumer from exposure to cyanotoxins. Consequently, drinking water treatment should remove cyanobacteria without compromising cell integrity in order to simultaneously eliminate intracellular toxins, but it should also remove potentially extracellular toxins previously released in raw water.

7.1. Overview of drinking water treatment

Drinking water treatment plants usually include a sequence of fundamental and optional processes, as presented in Fig. 6. The succession of these processes strongly differs according to the quality of the water resource but also according to the country and region. For example, while the most basic treatment for a high quality surface water resource would typically consist of coarse filtration followed by clarification to remove natural organic matter (NOM) and disinfection to inactivate pathogens, the decreasing quality of the in-coming surface water resource would require the application of additional processes to fulfill drinking water quality standards.

Water treatment processes are usually divided into 2 categories: those based on the retention of contaminants (clarification, adsorption, filtration…), and those based on the degradation of contaminants (UV irradiation, ozonation, chlorination…). While retention-based treatments generally require the regular application of cleaning procedure (backflush to limit fouling) as well as the replacement of pseudo-consumables (activated carbon and

membranes), degradation-based treatments may lead to the formation of potentially harmful known or unknown by-products such as trihalomethanes (THMs).

7.2. Impact of pre-treatment on cyanobacteria and cyanotoxins

Upon entering a water treatment plant, raw water is first coarsely filtered then potentially pre-oxidized. Both of these steps are often referred to as pre-treatments, as indicated in Fig. 6. Coarse filtration aims to remove macro-contaminants (leaves, plastic bags…) that could either damage treatment facilities or disturb following treatment processes. However, it does not greatly affect microcontaminants such as cyanobacteria and their toxins. The optional pre-oxidation by chlorine or ozone aims to improve the efficiency of next treatment steps, but it also damages the membrane of cyanobacteria (Miao and Tao, 2009). As indicated in Table 3, pre-oxidation induces cell lysis and the release of intracellular toxins. Also, the rapid consumption of chlorine and ozone by the high amount of dissolved organic carbon (DOC) in water at this stage of the treatment is likely to prevent substantial toxin oxidation. Consequently, while pre-oxidation is becoming less prevalent due to the production of harmful by-products, it should also be avoided when a bloom occurs in drinking water resources.

7.3. Impact of retention-based treatment on cyanobacteria and cyanotoxins

7.3.1. Coagulation-flocculation-sedimentation

The first steps in common drinking water treatment, coagulation-flocculation-sedimentation, aims to remove colloidal material (negatively charged suspended particles) in order to decrease turbidity. The addition of iron or aluminum salts neutralizes negative charges of colloids and prevents electrostatic repulsion between particles. Consequently,

colloids tend to agglomerate and form bigger particles (flocs) subsequently removed by sedimentation.

Cyanobacteria are microscopic microorganisms with negative charges on the membrane that can be roughly considered as colloids and removed by coagulation-flocculation-sedimentation. For example, up to 90% removal can be achieved on cultured *Microcystis* (Hall et al., 2000), but the dose of coagulant has to be increased according to the concentration of cyanobacteria in raw water and the organic matter content (Briley and Knappe, 2002; Velzeboer et al., 1995). Indeed, the higher concentration of cyanobacteria the more negative charges to be neutralized. In addition, the negative charges on the membranes increase with the production of polysaccharides during exponential growth. Consequently, the removal of cyanobacteria also depends on the age of the cells (Konno, 1993; Pieterse and Cloot, 1997).

Although coagulation-flocculation-sedimentation is capable of removing cyanobacteria, certain species containing gas vacuoles may disturb sedimentation by preventing flocs to settle (Pieterse and Cloot, 1997). Therefore, some studies showed that dissolved air flotation (DAF) could also efficiently remove cyanobacteria instead of sedimentation (Teixeira and Rosa, 2006a; Teixeira et al., 2010). In this case, the air injected at the bottom of the reactor carries the cells to the surface where they can be removed by scrapping.

Both sedimentation and DAF efficiently remove intracellular toxins since various studies have concluded that the elimination of cyanobacteria without damage to cell membrane or toxin release can occur (Table 3). However, once transferred into the sludge resulting from these processes, up to 90% of the cyanobacteria are lysed and released their toxins within 24 hours (Drikas et al., 2001). Therefore, sludge should be quickly extracted in order to avoid any back contamination of water by toxins diffusing to the aqueous phase.

On the contrary, coagulation-flocculation-sedimentation or DAF are not expected to remove extracellular toxins since both of them are designed to remove particles. As indicated in Table 3, this theory was confirmed by studies showing no difference in the concentration of MCs after treatment. The impact of these processes on other cyanotoxins was not further investigated.

7.3.2. Sand filtration

Slow sand filtration was shown to remove both cyanobacteria and their toxins during water treatment (Grützmacher et al., 2002). For example, 85% to 99% removal of MCs could be achieved when filtering water containing *Planktothrix agardhii* but the removal rate drastically decreases at low water temperature. While the main process for the elimination of MCs from a healthy cyanobacterial population was physical filtration of intracellular toxins (Grützmacher et al., 2002), extracellular toxins were also shown to be biodegraded (Bourne et al., 2006; Ho et al., 2006a; Ho et al., 2007). Indeed, the upper layer of sand filters potentially allows the growth of microorganisms. Among them, some bacteria brought in from the raw surface water resource could efficiently degrade MCs but a latency period might be required. With the isolation of microorganisms able to assimilate MCs (Ho et al., 2007; Ho et al., 2012a; Ho et al., 2012b) and the identification of the related genes (Bourne et al., 2001; Dziga et al., 2012; Yan et al., 2012), biodegradation offers a promising alternative for the removal of cyanotoxins in drinking water treatment. However, toxin-degrading microorganisms may not be able to grow in each sand filter of each drinking water treatment plant and biodegradation of cyanotoxins other than MCs have not been observed yet. Consequently, biodegradation of cyanotoxins on slow sand filters should not yet be considered as a remedial treatment by itself but as a link in the chain of a multi-barrier approach.

7.3.3. Membrane filtration

The term membrane filtration covers various processes characterized by the pore size of the associated membrane: microfiltration (0.1-10 µm), ultrafiltration (1-100 nm), nanofiltration (around 1 nm) and reverse osmosis (0.1 nm). These retention techniques have received considerable attention for their potential to remove microcontaminants in drinking water treatment. For example, according to the membrane employed, membrane filtration processes can efficiently remove cyanobacteria and their toxins, as indicated in Table 3.

Microfiltration and ultrafiltration processes are particularly efficient to remove cyanobacteria and intracellular toxins. For instance, both kinds of membranes were shown to achieve 98% removal of *Microcystis aeruginosa*, a toxic cyanobacteria frequently detected in drinking water resources (Chow et al., 1997). Although clogging and cell lysis are primary concerns in any filtration technique, damage to cell membranes were shown to be non-existent or limited during microfiltration and ultrafiltration, which prevents the increase of extracellular toxins in the permeate (Table 3). On the contrary, extracellular toxins are not expected to be removed by microfiltration membranes because of the pore size. Similarly, even though ultrafiltration membranes were shown to remove extracellular MCs (Lee and Walker, 2008), they may not be able to retain smaller toxins. On the other hand, both kind of filtration techniques can be applied to remove extracellular toxins previously adsorbed on powdered activated carbon (Campinas and Rosa, 2010a; Dixon et al., 2011a).

Theoretically, cyanobacteria should be efficiently removed by nanofiltration and reverse osmosis (lower pore size compared to ultrafiltration), but cells are not supposed to reach these processes. In fact, cyanobacteria are eliminated by previous treatments in order to avoid immediate clogging of these membranes. However, both nanofiltration and reverse osmosis are particularly efficient for the retention of extracellular toxins, as indicated in Table 3. For example, more than 95% removal could be observed for MC-LR and ANTX-a

(Teixeira and Rosa, 2006b) while 90-100% removal could be observed for CYL (Dixon et al., 2010; Dixon et al., 2011b). In addition, reverse osmosis was also shown to remove NODs (Vuori et al., 1997) but no published data are available concerning STXs, ANTX-a(s), BMAA, APTX or LBTX.

Although membrane filtration seems to be a promising option to remove both cyanobacteria and cyanotoxins during drinking water treatment, nanofiltration and reverse osmosis are complex as well as expensive methods. Their high retention potential often implies subsequent re-mineralization of the treated water. In addition, the cost associated with the energy required by such processes makes them unaffordable for small drinking water treatment units.

7.3.4. Activated carbon

In drinking water treatment, activated carbon is employed in two forms: powdered (PAC) to perform adsorption simultaneously with clarification, or granulated (GAC) to perform adsorption in percolation units. While activated carbon does not have any impact on cyanobacteria and intracellular toxins, it can be successfully applied to remove extracellular MCs, CYL, ANTX-a and STXs (Table 3).

The removal of cyanotoxins mostly depends on the kind of adsorbent employed (Donati et al., 1994; Huang et al., 2007; Newcombe and Nicholson, 2004). Indeed, when studying the adsorption of MC-LR on 8 activated carbons, adsorbents with the largest volume of mesopores (pore diameter in the range 2-50 nm) were shown to be the most efficient (Donati et al., 1994). However, other cyanotoxins may require other activated carbons. For example, since STXs are smaller than MCs, using microporous instead of mesoporous carbon is recommended for their removal (Newcombe and Nicholson, 2004).

Water quality also has a strong influence on the removal of cyanotoxins by activated carbon since NOM can compete with contaminants and limit their adsorption (Donati et al., 1994; Huang et al., 2007). This phenomenon clearly appears when comparing the adsorption isotherms of MC-LR in ultrapure water treated with fresh versus preloaded adsorbent, or MC-LR in ultrapure water versus surface water treated with fresh adsorbent (Lambert et al., 1996). Indeed, the adsorption of the toxin significantly decreases in surface water and when using preloaded activated carbon. Moreover, the isotherms obtained with surface water or previously used activated carbon exhibit an alteration of the slope indicating much lower adsorption capacity for toxin concentration below 0.15 µg/L. Therefore, although activated carbon efficiently retains MC-LR, reaching lower concentration would require a high and unusual amount of adsorbent for drinking water treatment (Lambert et al., 1996).

Activated carbon can also fix and grow specific microorganism and subsequently eliminate cyanotoxins by biodegradation (Newcombe and Nicholson, 2004). Therefore, after a latency period, the removal of cyanotoxins could progressively switch from adsorption to biodegradation.

While activated carbon can efficiently retain cyanotoxins, their complete adsorption would require a high amount of different adsorbent types, and their biodegradation on GAC may not necessarily occur in each drinking water treatment plant. Consequently, activated carbon should not be considered as an individual remediation measure but as a part of a multi-barrier approach.

7.4. Impact of degradation-based treatment on cyanobacteria and cyanotoxins

7.4.1. UV irradiation and photocatalysis

UV irradiation is a potential process for drinking water disinfection since light in the range 240-280 nm inactivates microorganisms by inducing DNA alteration. However,

increasing the UV dose can generate highly reactive hydroxyl radicals ($OH^{\bullet}$). Therefore, UV irradiation can also be employed as an advanced oxidation process (AOP) in order to remove organic contaminants. For this specific purpose, combining UV irradiation with ozone or hydrogen peroxide usually enhances the efficacy of the treatment by increasing $OH^{\bullet}$ production. Additionally, photocatalysis of trace contaminants by titanium dioxide (TiO_2) is another UV-based AOP that could potentially be applied in drinking water treatment although the formation of unknown and potentially toxic by-products remains an issue.

UV irradiation can potentially remove MCs, ANTX-a and CYL from drinking water but its effect on other cyanotoxins has not been investigated (Afzal et al., 2010; He et al., 2012; Kaya and Sano, 1998; Senogles et al., 2000a; Tsuji et al., 1995). The efficacy of such treatment depends on the lamp type and design, the intensity of the irradiation, the UV spectrum of each toxin and the turbidity of water. For instance, since MCs have a maximum absorbance at 240 nm, they can be transformed by a germicidal lamp emitting at 254 nm. When exposing MC-LR (10 mg/L in high purity water) to UV irradiation, toxin removal was shown to increase from 60% within 30 minutes to 100% within 10 minutes while the irradiation shifted from 147 $\mu W/cm^2$ to 2550 $\mu W/cm^2$ (Tsuji et al., 1995). As a result, 3 non-toxic by-products have been identified: 2 geometrical isomers of MC-LR consisting in a different conformation of the conjugated diene, plus another compound formed by addition between the benzene ring and one of the double bonds of the conjugated diene (Kaya and Sano, 1998). On the contrary, ANTX-a is not degraded using a low pressure disinfection UV lamp (ANTX-a does not absorb at 254 nm) but only using a medium pressure UV lamp (Afzal et al., 2010) with a broader UV emission spectrum.

For both MCs and ANTX-a, combining UV irradiation with the addition of hydrogen peroxide enhances the degradation of the toxin (Afzal et al., 2010; He et al., 2012; Qiao et al., 2005). While pH in the range of 7-8 was found to be optimal for MC-RR degradation,

increasing H_2O_2 over 1 mM and UV irradiation over 3.66 mW/cm^2 would not further increase the degradation rate (Qiao et al., 2005). Indeed, the production of $OH^{\bullet}$ tends to increase with the concentration of hydrogen peroxide but, at some point, H_2O_2 itself consumes hydroxyl radicals and competes with water contaminants. Besides, toxin removal is also correlated with water quality since $OH^{\bullet}$ will also react with DOC (He et al., 2012). Similar results were observed for ANTX-a, and the medium pressure UV lamp necessary for toxin elimination by standalone UV irradiation can be replaced by a common low pressure UV lamp (Afzal et al., 2010).

Adding a photocatalyst and increasing pH was shown to improve the efficiency of UV irradiation to remove cyanotoxins (Senogles et al., 2001). For instance, the half-life of CYL decreases from 14 min with UV irradiation alone to less than 2 min with UV irradiation in presence of TiO_2 (Senogles et al., 2001). In addition, photocatalysis by TiO_2 also enhances the transformation of NODs and MCs (Lawton et al., 1999; Liu et al., 2005) but, unlike CYL, acidic conditions are preferable (Antoniou et al., 2008). While numerous by-products and intermediates have been identified (Antoniou et al., 2008), it is considered that the transformation of NODs and MCs mainly occurs through isomerization and subsequent attack by $OH^{\bullet}$ leading to substitution and cleavage of the Adda amino acid (Liu et al., 2005; Liu et al., 2009), which is consistent with the lower toxicity of treated samples (Lawton et al., 1999; Liu et al., 2005).

7.4.2. Ozonation

Ozone can potentially be used as a disinfection process to produce drinking water. However, since this powerful oxidant is not persistent in water, it is mostly used to remove trace organic contaminants by chemical degradation. For this purpose, ozone can also be used

along with H_2O_2 or Fe(II), which generates more $OH^{\bullet}$ and usually enhances the degradation of chemicals.

As indicated in Table 3, ozone reacts with all the common cyanotoxins but less efficiently with STXs. For instance, at pH 7 and 20°C, 5 mg/L MC-LR in high purity water can be completely removed by 2 mg/L O_3 within 2 minutes (Al Momani and Jarrah, 2010). Moreover, the reaction kinetic was shown to improve when decreasing pH as well as increasing ozone dose with temperature (Al Momani et al., 2008; Al Momani and Jarrah, 2010; Shawwa and Smith, 2001). While the occurrence of NOM in the sample is known to limit toxin removal by competing for O_3, it is usually considered that the ozone dose necessary to achieve a 0.05 mg/L residual ensures the complete removal of MCs (Brooke et al., 2006; Newcombe and Nicholson, 2004). In fact, ozonation mainly alter MCs through initial $OH^{\bullet}$ attack on the conjugated diene of the molecule while further oxidation leads to the cleavage of the Adda amino acid and the opening of the peptide ring (Al Momani and Jarrah, 2010; Miao et al., 2010). Such alteration of the toxin, particularly the Adda moiety, is consistent with the decrease or total elimination of toxicity in ozonated samples analyzed either by mouse bioassays or PPIA (Brooke et al., 2006; Miao et al., 2010).

Similar efficacy (with similar impact of temperature and DOC) has been observed with CYL and ANTX-a but by-products and residual toxicity have not been considered (Al Momani, 2007; Rodríguez et al., 2007a). However, while the removal of ANTX-a is improved when pH increases (unlike MCs), STXs were shown to be poorly altered by ozonation (Newcombe and Nicholson, 2004).

The use of ozone either with H_2O_2 or Fe(II) for the removal of cyanotoxins has not been extensively studied. However, both O_3/H_2O_2 and O_3/Fe(II) where shown to enhance the transformation of MCs and ANTX-a compare to O_3 alone (Al Momani, 2007; Al Momani et

al., 2008). Further research could be undertaken to determine if O_3/H_2O_2 and O_3/Fe(II) may also enhance the degradation of the recalcitrant STXs.

7.4.3. Chlorination and chloramination

Chlorination is frequently used to perform drinking water disinfection. Chlorine is often preferred to ozone or UV irradiation since it is persistent and can prevent the contamination of drinking water by pathogens in the distribution network. Similarly, in water treatment facilities, chlorine can also be replaced by chloramine or chlorine dioxide. However, chlorine and its substitutes are also oxidants that can react with water contaminants.

A recent review on the chlorination of cyanotoxins indicates that MCs and NODs as well as CYL and STX are quickly transformed, but the kinetic was too slow for ANTX-a to be altered during drinking water treatment (Merel et al., 2010a). Although MCs are highly reactive with chlorine, the alteration of these toxins decreases when pH and DOC increase. Indeed, increasing pH will progressively transform chlorine into ClO^- (a weaker oxidant prevailing at pH above 8) while DOC will compete with the toxin. It is usually considered that MCs are efficiently transformed when pH is maintained below 8 and chlorine dose is enough to ensure 0.5 mg/L residual after 30 minutes (Acero et al., 2005; Newcombe and Nicholson, 2004; Nicholson et al., 1994). For instance, with chlorine 20 times in excess at pH 7, more than 99% of MC-LR in high purity water can be transformed within 5 minutes (Merel et al., 2009). Similar efficacy is also expected in water treatment plants since for the worst scenario (high toxin concentration with only chlorine residual, respectively 20 µg/L and 0.5 mg/L) chlorine would be at least 350 times in excess compare to MCs (Merel et al., 2009). Additionally, chlorination of MCs leads to the formation of numerous by-products through multiple hydroxylation or chlorine substitution on the initial toxin, including on the Adda moiety (Merel et al., 2009; Tsuji et al., 1997). However, multiple studies based on bioassays

or PPIA have reported a decrease in toxicity of the mixture after chlorination (Merel et al., 2010a; Nicholson et al., 1994; Rodríguez et al., 2008; Tsuji et al., 1997).

Various studies indicate that CYL is quickly transformed during chlorination (Banker et al., 2001; Merel et al., 2010b; Newcombe and Nicholson, 2004). For instance, with chlorine 10 times in excess at pH 7 and 20°C, more than 98% of CYL can be transformed within 2 minutes (Merel et al., 2010b). While neutral pH was found to be optimum (unlike MCs), toxin degradation was shown to occur twice faster when temperature increases from 10°C to 30°C (Rodríguez et al., 2007b). Although only 3 by-products formed by chlorine addition followed by cleavage of the uracil moiety have been identified, these were not shown to be toxic (Banker et al., 2001; Merel et al., 2010b). Only one study reported liver injuries in 40% of male mice when feeding the entire population with chlorinated cell free extract of C. raciborskii (Senogles-Derham et al., 2003). However, the ratio chlorine/CYL was not specified and chlorination is still usually considered to decrease the toxicity of the mixture.

Chlorination of STXs has not been extensively studied but some variants were shown to be efficiently transformed under specific conditions (Nicholson et al., 2003). Unlike MCs and CYL, toxin alteration improves from 20% to 98% when pH increases from 4 to 9. However, toxin alteration also depends on the variant. In fact, the following vulnerability has been established: GTX5 = dcSTX > STX > GTX3 = C2> C1> GTX2 (Nicholson et al., 2003). Although several chlorination by-products remain unknown, chlorination is considered to decrease the toxicity of the mixture since no acute toxicity could be observed by mouse bioassay (Nicholson et al., 2003). However, further research should be undertaken to confirm these results.

The use of ClO_2 and monochloramine to remove cyanotoxins has not been extensively studied. In fact, ClO_2 also efficiently transforms MC-LR and decreases the toxicity of the mixture but its efficiency with other cyanotoxins is limited or unknown. For example, CYL

has a 14.4 hour half-life when exposed to chlorine dioxide but a 1.7 minute half-life when exposed to chlorine (Rodríguez et al., 2007a). Similarly, monochloramine is a weak oxidant and reacts slowly with cyanotoxins. Indeed, even if monochloramine potentially reacts with CYL (Banker et al., 2001), the kinetic constant is 2400-fold lower than with chlorine in similar conditions (Merel et al., 2010a; Rodríguez et al., 2007b). Consequently, chloramination is considered inappropriate to remove harmful metabolites from cyanobacteria during drinking water treatment.

8. Regulation of cyanotoxins

The elaboration of a new regulation for cyanotoxins or any other chemical requires extensive scientific background established from multiple research programs across several disciplines. Indeed, as shown in Fig. 7, each regulating agency must consider multiple factors in order to define meaningful but applicable values for regulation. While cyanotoxins include multiple compounds, the first step of the regulation process consists in determining which toxins must be considered, individually or as a mixture. After selecting a suitable analytical method, the second step should estimate the occurrence of the selected toxins in the environment in terms of frequency and concentration. Then, human exposure can be assessed depending on the type of water and the maximum toxin concentration proposed from toxicological studies. However, the limit or threshold value should remain economically achievable with the technology currently available.

Toxic cyanobacterial blooms in surface water represent a growing public health concern due to the multiple sources of human exposure to toxins. Therefore, more than a decade after WHO's guideline for MC-LR, several countries already regulated cyanotoxins (Table 4) in drinking water and others like USA are also considering it. MCs are the most regulated cyanotoxins and most of the countries consider the 1 µg/L guideline as the

maximum concentration. However, in most cases this value does not apply to MC-LR only but to the sum of all MCs. Some countries also regulate CYL and STXs but while the maximum concentration for STXs is identical at 3 µg/L, the maximum concentration for CYL varies from 0.1 to 15 µg/L. New Zealand is the only country regulating MCs, NOD, CYL, ANTX-a, homoANTX-a, ANTX-a(s) and STXs. However, emerging cyanotoxins like BMAA, APTX or LTX are not considered, probably because the lack of data did not allow the calculation of a guideline.

Cyanobacterial toxins are also considered by several countries in recreational waters but only MCs are attributed specific values. As expected the maximum concentrations are higher than those defined for drinking water since the exposure is lower in terms of frequency (occasional for bathing but chronic for drinking water) and volume of water ingested. Dermatotoxins to which exposure is expected to be significant in recreational water are not considered, probably due to the lack of toxicological data available. In fact, the cell count appears to be the dominant parameter monitored to assess the quality of recreational water.

Analyzing water for toxins is expensive and it cannot be done on a regular basis on every water body. Therefore, most of the countries regulating cyanotoxins have developed different risk management schemes with several alert levels based on cell counts. For example, the World Health Organization has proposed 2 alert levels, 2 for 20,000 cells/ml and 3 for 100,000 cells/ml, this last leading to bathing prohibition (Chorus and Mur, 1999). The density of cyanobacteria in water usually triggers the analysis of cyanotoxins which can lead to administrative measures ranging from public information to restriction or interdiction of recreational activities. However, more research and feedback from policy makers is needed to improve regulation of cyanotoxins and protect human health without worsening the economic impact of blooms.

9. Economic impact of cyanobacterial blooms

Cyanobacterial blooms in surface water may have a strong impact on local economies. For example, the restriction or interdiction of recreational activities such as bathing or shellfish harvesting in contaminated water can be detrimental to tourism. During the 1991 bloom of neurotoxic *Anabaena* which occurred in the Darling River, Central Australia, losses to the tourism industry were estimated around $1.5 million (Steffensen, 2008). In the same period, because of another bloom occurring in the Hawkesbury Nepean River, New South Wales, Australia, revenues of tourism facilities were estimated to be $6.7 million lower compared to the previous year (without a bloom). Considering that the bloom was not toxic, the entire loss of revenues was only the consequence of a negative publicity (Steffensen, 2008). Multiple blooms over a wide range of latitudes can have a significant impact on the tourism industry but the associated cost is not often calculated.

Preventive and remedial measures also contribute to the global economic impact of cyanobacterial blooms. For example, preventing cyanobacterial growth by artificial water circulation in a lake or reservoir requires the installation of various pumps at specific locations and regular maintenance. Applying algaecides to eradicate an existing bloom is also associated with an elevated cost. Indeed, in Australia, SA Water spends over $1 million a year to apply algaecides and dispose of the copper contaminated water treatment sludge (Steffensen, 2008). In order to prevent blooms by inhibiting photosynthesis, the same company also covered three storage reservoirs for a total cost of over $7 million.

Once a bloom appears in surface water, monitoring the evolution of the phenomenon implies the repetition of expensive analysis, sometimes over several months. For example, toxicity tests may cost over $1,000 per sample and monitoring several sampling sites may be necessary if a lake or reservoir is used for both recreational activities and drinking water

production. Therefore, in Australia, the cost estimation of bloom monitoring reaches over $8 million per year (Steffensen, 2008).

As indicated in Fig. 8, the global economic impact of cyanobacterial blooms should also include costs related to water treatment, health consequences and research programs. Indeed, while cyanobacterial blooms disturb drinking water production by increasing the consumption of reactants, they also decrease the productivity through faster filter clogging. Health consequences through human intoxication also represent a high expense, particularly in the case of acute (liver failure) or long term (cancer or neurodegenerative diseases) exposure. However, since moderate human intoxication is often limited to gastro-enteritis, a symptom common to numerous pathologies, patients are treated without considering the underlying cause and the relation with cyanotoxins is not or rarely established. Research programs and conferences should also be held frequently so that the most current science available can be used to alleviate the problem of cyanobacterial blooms in surface waters, but these activities and events have an associated cost as well.

The overall impact of cyanobacterial blooms on the economy remains poorly understood. Therefore, specific research is required on this topic with particular emphasis on public health consequences and water treatment in the frame of global warming.

10. Research needs

The previous sections established a broad state of the art on harmful cyanobacterial blooms, considered their occurrence, toxicity, management and economic impact. However, several research needs still exist.

10.1. History of bloom occurrence and related environmental conditions

In most surface waters, long-term, quantitative data regarding phytoplankton and cyanobacteria assemblage changes over time is non-existent. Paleolimnological techniques can be applied to re-construct past histories of lakes and reservoirs by collecting and analyzing sediment cores. Buried within the sediments of lakes and reservoirs is a repository of proxy information that can be used to construct trophic state and environmental changes over time. Information contained within sediment cores can also be used to predict past climates, vegetation changes within the watershed, nutrient loading and recycling, etc. Sediment cores can be age-dated using a variety of techniques including stable isotopes, varve formation, etc. They can also be analyzed for algal and cyanobacterial pigments. By doing so, it is possible to recreate past instances of bloom formation and the environmental circumstances leading to their occurrence. Consequently, such historical data accumulated over a period of time beyond human scale would greatly benefits the development of new and more accurate predictive models for bloom occurrence.

10.2. Prediction of bloom occurrence

Predicting the occurrence of cyanobacterial blooms remains challenging. Cyanobacteria and phytoplankton in general are very patchy assemblages that exhibit great temporal and spatial variability. Many species of cyanobacteria exist and each has different environmental requirements for survival and growth. For example, some species contain heterocysts and are capable of “fixing” atmospheric nitrogen, some are filamentous and some colonial (sometimes forming macroscopic colonies), some produce copious amounts of exopolysaccharides, etc. Cyanobacteria are not a homogenous group of organisms and show great diversity between species. This fact makes bloom prediction and management difficult. While predictive models need to be established, refinement based upon predominant and

problematic species as well as individual ecosystems and environmental conditions can be incorporated within a generalized framework.

Future research should also address the impact of climate change on bloom occurrence. Climate change may increase the frequency and magnitude of blooms and alter toxin distribution by cyanobacteria spreading to latitudes outside of their current range. Increased runoff and altered hydrologic regimes may increase nutrient concentration in surface waters, favoring the occurrence of cyanobacterial blooms and overall eutrophication. Generalized and predictive models that address the overall spread or re-distribution of cyanobacteria, and potential bloom formation and toxin formation, due to climate change are needed.

10.3. Prediction of toxicity

The occurrence of cyanobacterial blooms in surface water does not necessarily imply toxicity. Although it is clearly established that the ability of cyanobacteria to perform the biosynthesis of harmful metabolites depends on their gene pool, the activation of these genes by environmental factors remains poorly understood. Therefore, future research projects should particularly focus on determining the environmental conditions needed for biosynthesis of toxin on a species-by-species basis. Although this is a daunting task, the time to begin building such a repository of information is now. If such information could be incorporated into future or existing models, it would be a very valuable tool capable of predicting blooms toxicity on a localized basis at least.

10.4. Bloom monitoring

Blooms of cyanobacteria occurring in surface waters need to be accurately monitored due to their potential toxicity. Therefore, more efficient and affordable monitoring strategies

should be developed for both cyanobacteria (identification and enumeration) and their toxins (identification and quantification). Particularly, further development of in situ monitoring probes should be a priority. The automation and adaptation of molecular techniques for in situ application (e.g., onsite PCR) would offer the capability for constant, real-time monitoring of for potentially toxic cyanobacterial species. Similarly, automation and adaptation of ELISA methods for onsite remote toxin analysis would be a major breakthrough in bloom monitoring.

Besides in situ technique development, laboratory analysis of cyanotoxins also requires further research in order to develop rapid and reliable methods for simultaneous quantification of multiple compounds. Improvement of LC-MS or immunological methods would significantly decrease the cost of analysis and improve bloom monitoring.

While blooms are carefully monitored in drinking water resources or surface waters used for recreational activities, quantifying their occurrence often remains difficult. Indeed, such phenomenons are often managed by local water authorities and are not reported into any official national or international database. Consequently, significant amount of information may remain inaccessible to scientists who have to rely on published literature, probably describing a very low percentage of the blooms monitored worldwide. In addition, numerous blooms occurring in water that are not submitted to specific control, such as private ponds, are not even reported to local agencies. Therefore, both the occurrence of cyanobacterial blooms and their potential health effects may be considerably underestimated. Consequently future research should also be undertaken to address this concern.

10.5. Bloom and drinking water treatment

The present state of the art on the fate of cyanotoxins during drinking water treatment clearly indicates that any individual toxin can be efficiently removed or transformed by at

least one process. However, no treatment process has been proven to simultaneously remove or transform all the cyanotoxins. For instance, although chlorination can transform MCs, CYL and STXs, the optimum pH is significantly different and chlorination of drinking water cannot ensure the complete transformation of these toxins in a mixture. Consequently, the elimination of cyanotoxins during drinking water treatment must be based on a multibarrier approach. Therefore, future studies should focus on the removal of a mixture of toxins (including less common ones such as ANTX-a(s) or BMAA for which no data are currently available) through several treatment processes in series. However, while the concept of multibarrier should not be difficult to implement for major water treatment facilities, it may not be affordable for small scale units delivering water to a low amount of consumers. In this context, some alternative measures, such as toxin removal by home filters, should also be considered.

10.6. Regulation and economic impact

Cyanotoxins might be potential candidates for future regulation in drinking water. For example, ANTX-a, CYL and MC-LR were on the third contaminant candidate list released by the US EPA. Therefore, future research should investigate the suitability of regulating cyanotoxins, considering occurrence and toxicological data but also analytical capabilities and economic implications. Indeed, an inappropriate regulation of cyanotoxins in drinking water could lead to significant expenses (modification of treatment plants, monitoring programs…) sometimes unaffordable for small communities.

Blooms of cyanobacteria are often associated with a restriction of recreational activities and direct consequences on tourism. While some examples have been studied in Australia to assess the local economic loss, the overall economic impact of blooms remains unknown. As presented in this review, future research should also estimate the cost of blooms

considering preventive and remedial measures, monitoring and research programs, water treatment and health consequences.

11. Conclusion

Cyanobacteria are widespread microorganisms, naturally occurring in most of surface water. It is well established that high concentration of nutrients is one of the most important factor leading to their excessive development. According to the predominant strains, some blooms are potentially harmful. Indeed, the gene pool of cyanobacteria determines their ability to perform the biosynthesis of a wide range of toxins categorized into hepatotoxins, neurotoxins and dermatotoxins. While the biosynthesis pathways have been identified for most of them, the activation of the related genes by environmental factors and therefore the conditions leading to toxin production remain unknown.

Humans are potentially exposed to harmful algal blooms through recreational activities in contaminated water and through drinking water produced from contaminated resources. Therefore, numerous strategies have been tested to prevent or eliminate blooms of cyanobacteria. While water recirculation appears to be successful in the short term, a long term and sustainable strategy consist in reducing the introduction of nutrients in surface waters. Eradication of an occurring bloom by algaecides should be avoided because of the subsequent release of toxins.

In order to protect consumer's health, drinking water treatment has to remove both intracellular and extracellular toxins. While the usual clarification and membrane filtration efficiently removes cyanobacteria and intracellular toxins, combining ozonation and chlorination should ensure the removal of the most common extracellular toxins. However, although individual toxins can be efficiently removed or transformed by at least one treatment step during the production of drinking water, no treatment has been proven to simultaneously

remove all the cyanotoxins in a mixture. Therefore, the efficient management of cyanotoxins in drinking water treatment must be based on a multibarrier approach.

Even though cyanobacteria and their toxins have been extensively studied, further research is required to address several gaps in the actual state of knowledge. For instance, there is a major need for models predicting the occurrence of blooms but also their potential toxicity. In addition, the impact of a changing climate on blooms also needs to be addressed. Moreover, while the fate of common cyanotoxins during water treatment is already documented, the fate of uncommon toxins such as BMAA, APTXs or LTXs remains completely unknown. Besides, the occurrence of toxic cyanobacteria is also a major research topic for economic sciences in order to assess the impact of blooms on local economies.

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Figure Captions

Table Captions

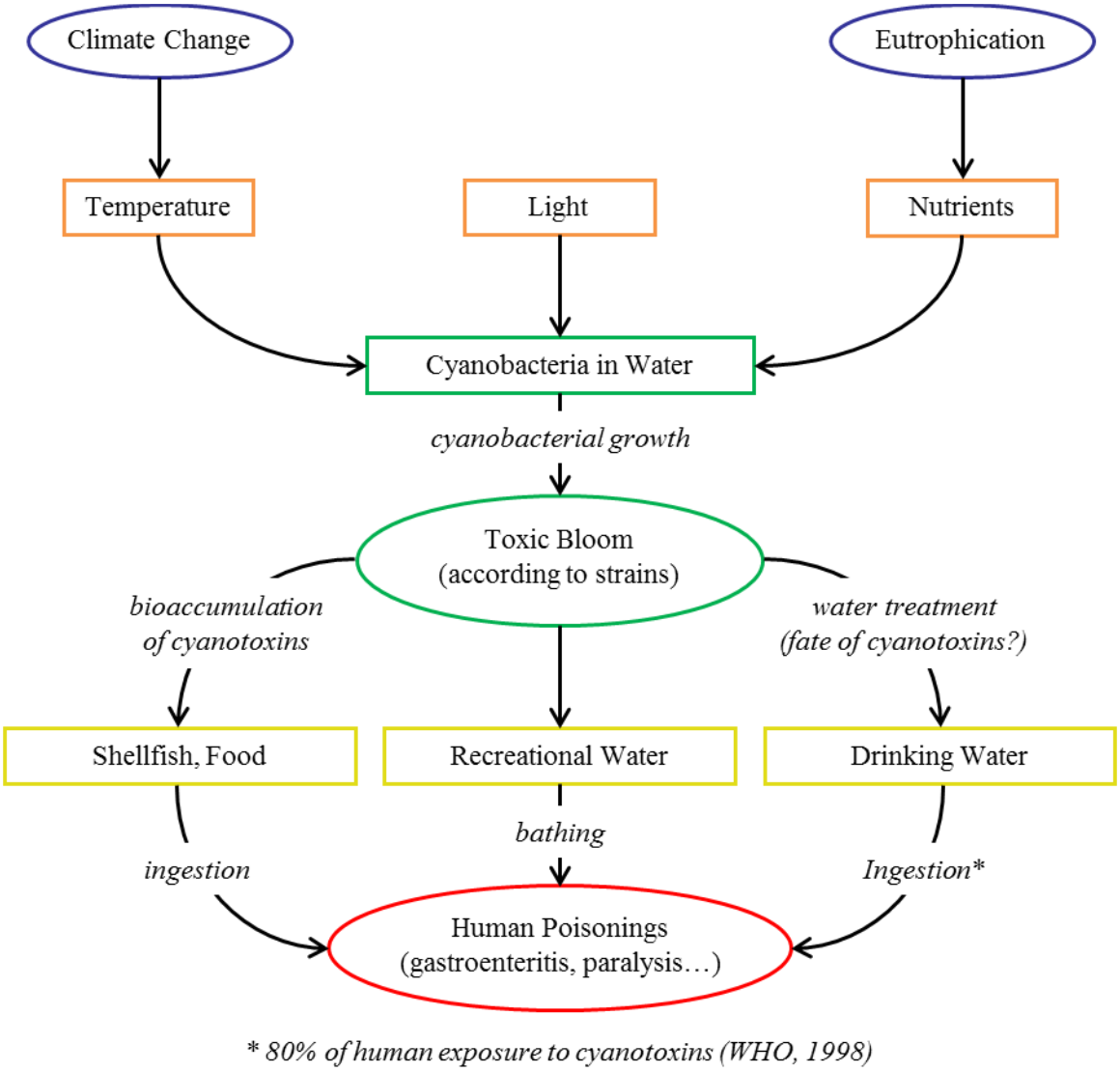


Fig. 1: Origin of toxic cyanobacterial blooms and human exposure.

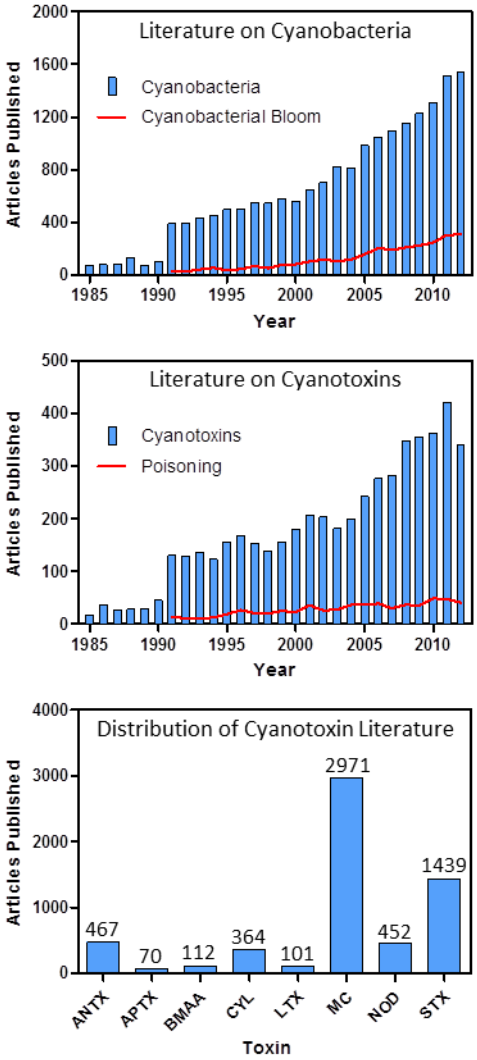
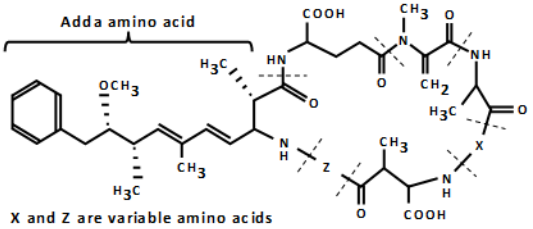


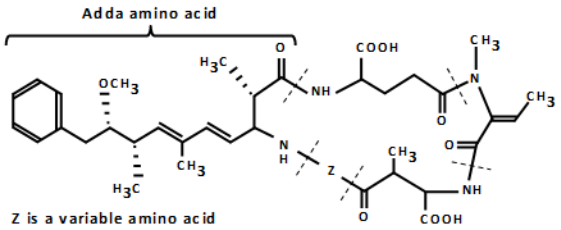
Fig. 2: Overview of literature available on cyanobacteria and cyanotoxins.

Cyanobacterial Hepatotoxins

Microcystin



Nodularin



Cylindrospermopsin

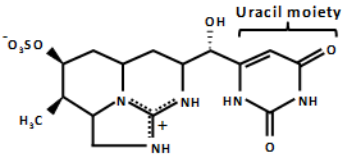
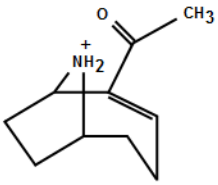


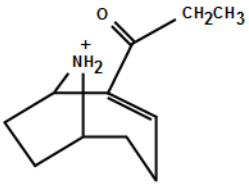
Fig. 3: Structure of cyanobacterial hepatotoxins.

Cyanobacterial Neurotoxins

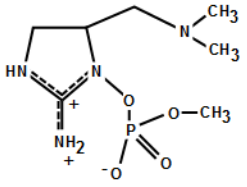
Anatoxin-a



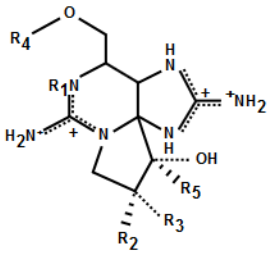
Homoanatoxin-a



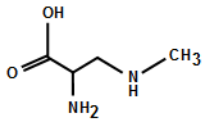
Anatoxin-a(s)



Saxitoxin



β-methylamino-L-alanine



Toxin	R_1	R_2	R_3	R_4	R_5
STX	H	H	H	$CONH_2$	OH
GTX2	H	H	OSO_3^-	$CONH_2$	OH
GTX3	H	OSO_3^-	H	$CONH_2$	OH
GTX5	H	H	H	$CONHSO_3^-$	OH
C1	H	H	OSO_3^-	$CONHSO_3^-$	OH
C2	H	OSO_3^-	H	$CONHSO_3^-$	OH
C3	OH	H	OSO_3^-	$CONHSO_3^-$	OH
C4	OH	OSO_3^-	H	$CONHSO_3^-$	OH
neoSTX	OH	H	H	$CONH_2$	OH
GTX1	OH	H	OSO_3^-	$CONH_2$	OH
GTX4	OH	OSO_3^-	H	$CONH_2$	OH
GTX6	OH	H	H	$CONHSO_3^-$	OH
dcSTX	H	H	H	H	OH
dcneoSTX	OH	H	H	H	OH
dcGTX1	OH	H	OSO_3^-	H	OH
dcGTX2	H	H	OSO_3^-	H	OH
dcGTX3	H	OSO_3^-	H	H	OH
dcGTX4	OH	OSO_3^-	H	H	OH
LWTX1	H	OSO_3^-	H	$COCH_3$	H
LWTX2	H	OSO_3^-	H	$COCH_3$	OH
LWTX3	H	H	OSO_3^-	$COCH_3$	OH
LWTX4	H	H	H	H	H
LWTX5	H	H	H	$COCH_3$	OH
LWTX6	H	H	H	$COCH_3$	H

Fig. 4: Structure of cyanobacterial neurotoxins.

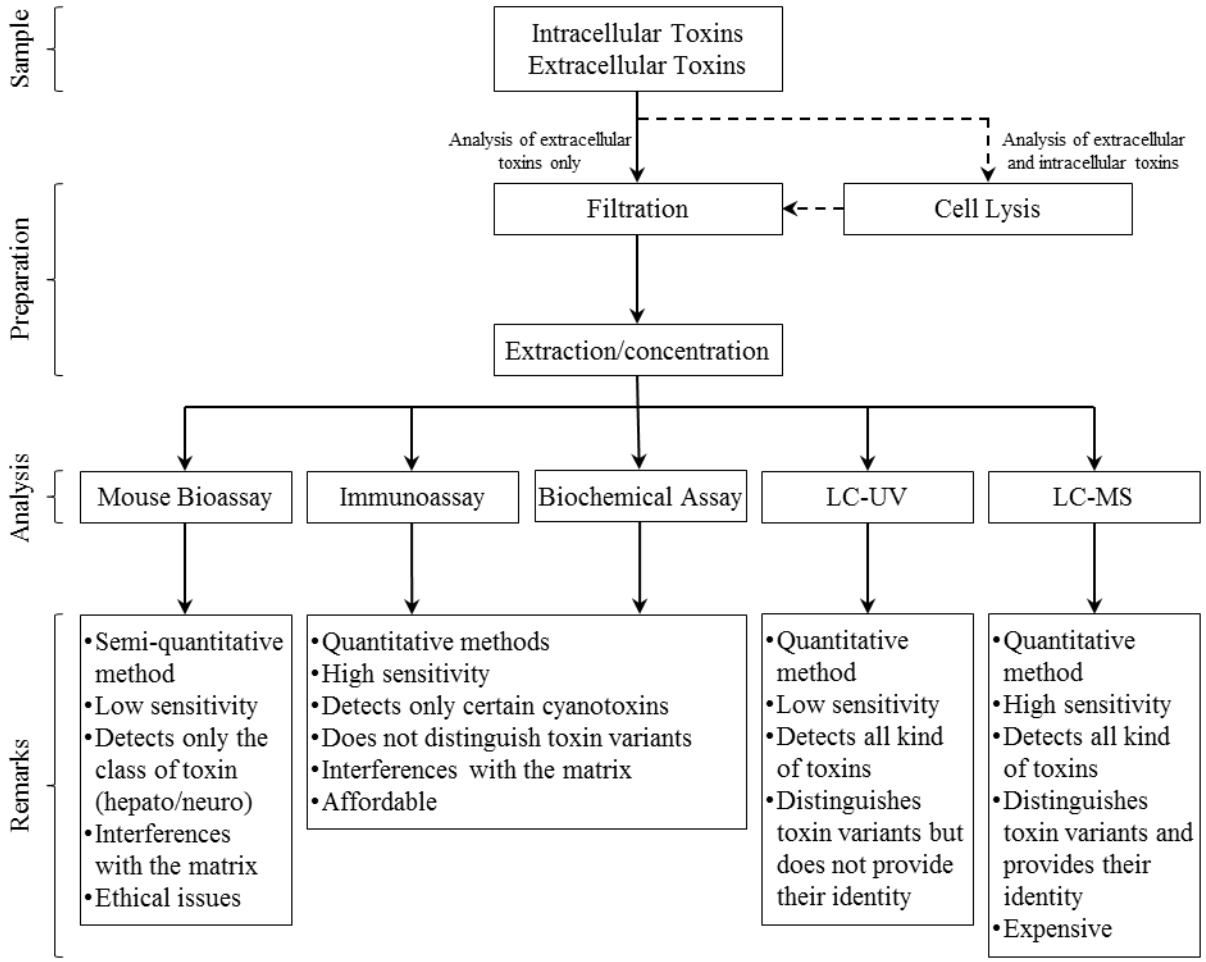


Fig. 5: Overview of sample preparation and analytical methods for the detection of cyanotoxins.

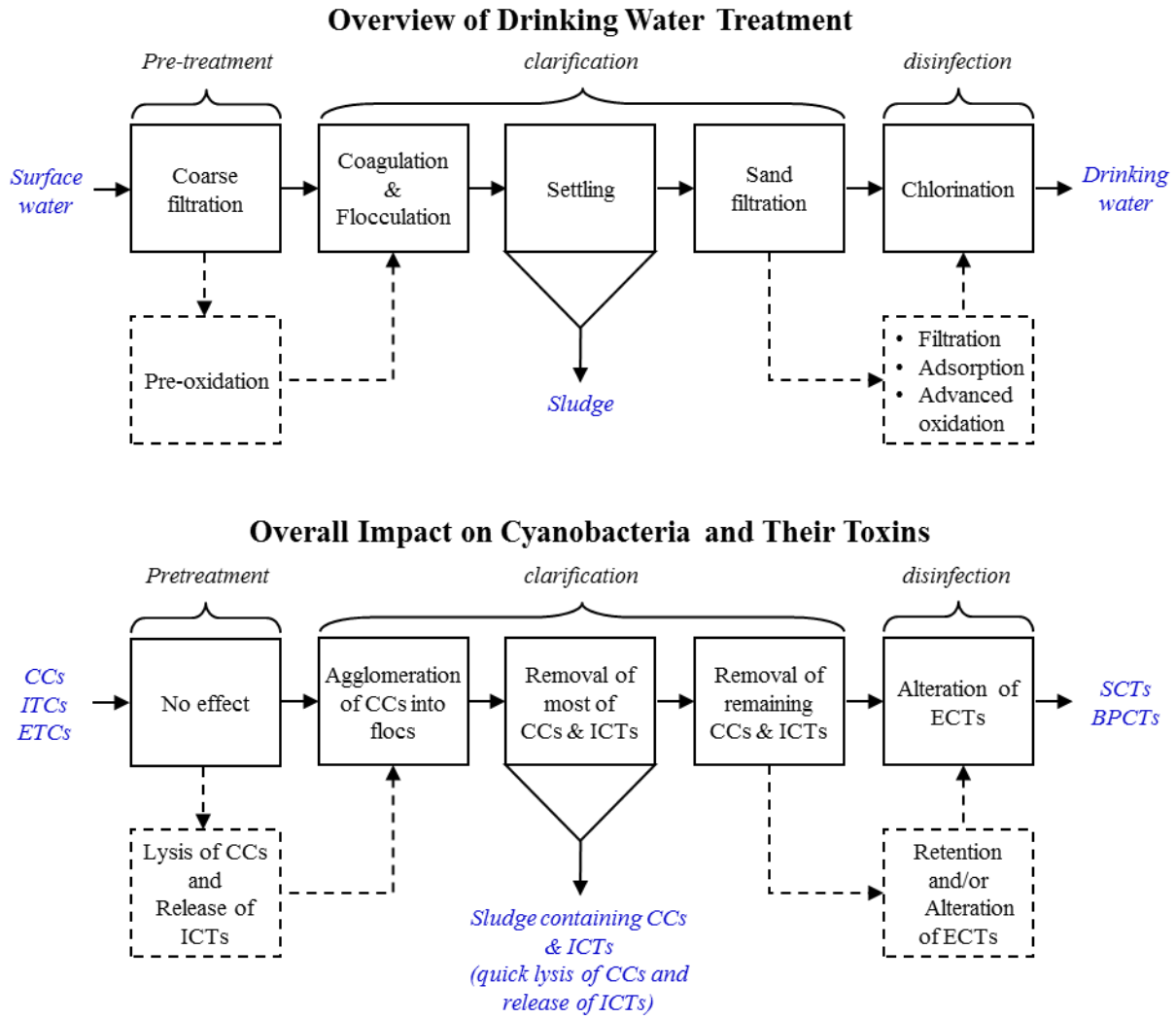


Fig. 6: Overview of drinking water treatment and the overall impact on cyanobacteria and cyanotoxins.

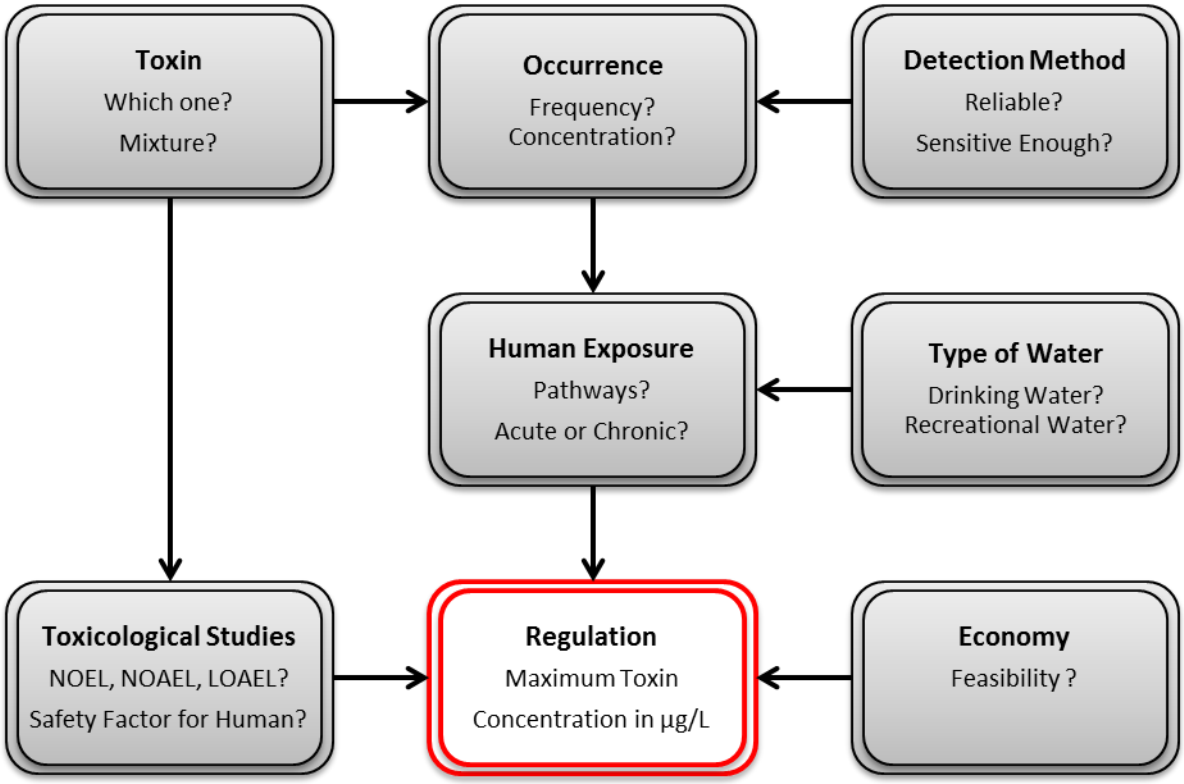


Fig. 7: Parameters to consider when building a new regulation for cyanotoxins in water.

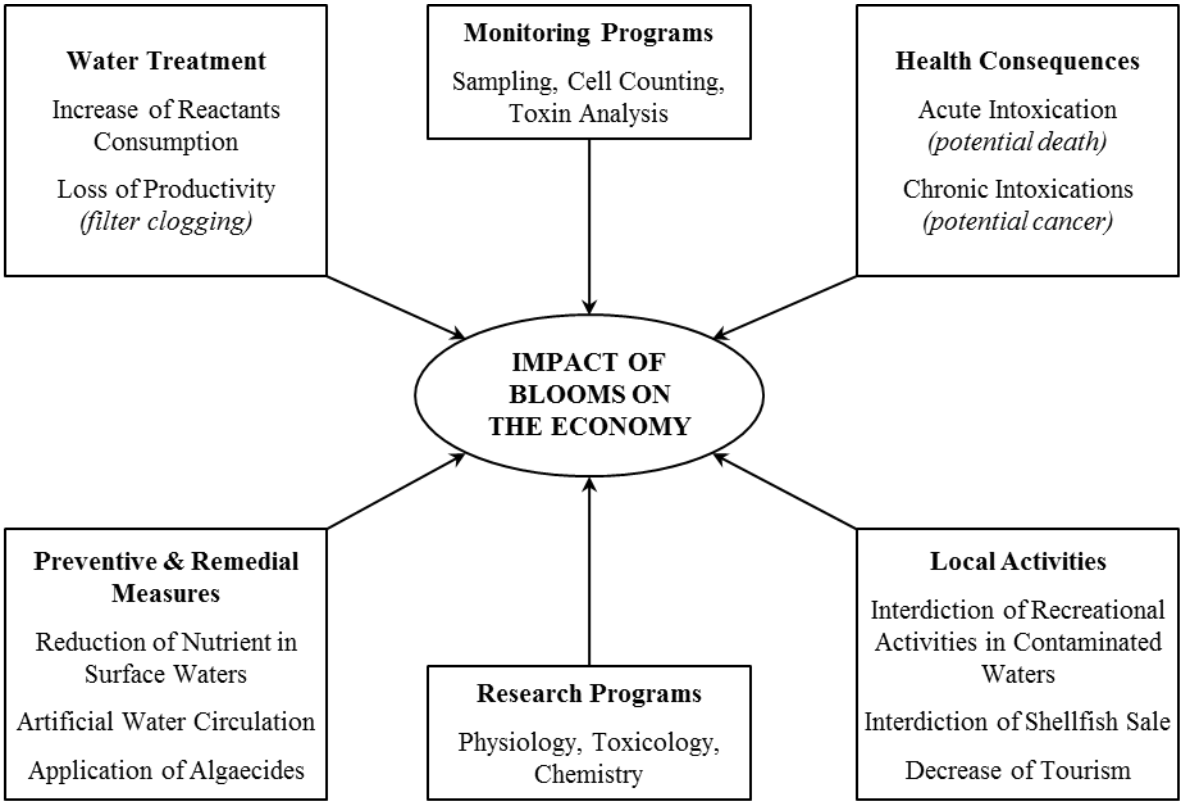


Fig. 8: Aspects to consider when assessing the economic impact of blooms.

Table 1: Toxicological characteristics of cyanotoxins.

Toxin	Mode of action[a]	Main effect[a]	LD_{50} (µg/kg)[a,b]
MCs	*Inhibit protein phosphatase*	*Liver failure and hepatic haemorrhage*	*25-150 (for the most toxic)*
NODs	*Inhibit protein phosphatase*	*Liver failure and hepatic haemorrhage*	*30-70*
CYL	*Inhibits protein synthesis*	*Liver and kidney failure*	*2100*
ANTX-a	*Binds to nicotinic acetylcholine receptors*	*Muscular paralysis*	*375*
ANTX-a(s)	*Inhibits acetylcholinesterase*	*Muscular weakness, dyspnea and convulsions*	*31*
STXs	*Bind to sodium channels*	*Ataxia, convulsions and paralysis*	*10 (for the most toxic)*
BMAA	*Binds to glutamate receptors*	*Neurodegenerative syndrome*	*Not specified*
APTXs	*Activate protein kinase C*	*Tumour promotion and skin irritation*	*Not specified*
LTXs	*Activate protein kinase C*	*Tumour promotion and skin irritation*	*250 (immature mice)*

[a] *van Apeldoorn et al., 2007* [b] *After intraperitoneal injection into mice*

Table 2: Literature survey of analytical methods for the detection of cyanotoxins.

Toxins	Immunological assay	Biochemical assay	LC-UV	LC-fluorescence	LC-MS	GC-MS	Other
MCs & NODs	1	2	3	4	5	6	7
CYL	8		9		10		11
ANTX-a			12	13	14	15	16
ANTX-a(s)		17			18		
STXs	19			20	21		22
BMAA			23	24	25	26	27

1) An and Carmichael, 1994; Carmichael and An, 1999; Humpage et al., 2012; Kaushik and Balasubramanian, 2012; Lawrence et al., 2001; Lawton and Edwards, 2008; Lawton et al., 2010; Lindner et al., 2004; Mathys and Surholt, 2004; McElhiney and Lawton, 2005; Metcalf et al., 2000; Msagati et al., 2006; Rapala et al., 2002; Spoof et al., 2003; Tillmanns et al., 2007; Triantis et al., 2010
2) Almeida et al., 2006; An and Carmichael, 1994; Bouaïcha et al., 2002; Carmichael and An, 1999; Heresztyn and Nicholson, 2001; Kaushik and Balasubramanian, 2012; Lawrence et al., 2001; Lawton and Edwards, 2008; McElhiney and Lawton, 2005; Msagati et al., 2006; Ortea et al., 2004; Rapala et al., 2002; Robillot and Hennion, 2004; Triantis et al., 2010; Ward et al., 1997; Wirsing et al., 1999
3) Aranda-Rodriguez et al., 2003; Bateman et al., 1995; Gurbuz et al., 2009; Kaushik and Balasubramanian, 2012; Kaya et al., 2001; Lawton et al., 1994; Lawton and Edwards, 2008; Mathys and Surholt, 2004; McElhiney and Lawton, 2005; Meriluoto, 1997; Metcalf et al., 2000; Ortea et al., 2004; Rapala et al., 2002; Spoof and Meriluoto, 2002; Spoof et al., 2003; Spoof et al., 2009; Spoof et al., 2010; Tillmanns et al., 2007; Triantis et al., 2010; Ward et al., 1997; Wirsing et al., 1999
4) Harada et al., 1997; Kaushik and Balasubramanian, 2012; Lawton and Edwards, 2008; Sano et al., 1992
5) Aranda-Rodriguez et al., 2003; Barco et al., 2002; Bateman et al., 1995; Chen et al., 2012; Cong et al., 2006; Dell'Aversano et al., 2004; del Campo and Ouahid, 2010; Diehnelt et al., 2005; dos Anjos et al., 2006; Frias et al., 2006; Herrmann et al., 2012; Kaushik and Balasubramanian, 2012; Kaya et al., 2001; Krüger et al., 2009; Lawrence et al., 2001; Lawton and Edwards, 2008; McElhiney and Lawton, 2005; Meriluoto, 1997; Msagati et al., 2006; Neffling et al., 2009; Oehrle et al., 2010; Ortea et al., 2004; Shan et al., 2011; Spoof et al., 2001; Spoof et al., 2003; Triantis et al., 2010; Wang et al., 2007b; Yuan et al., 1999; Zhang et al., 2004
6) Kaushik and Balasubramanian, 2012; Kaya and Sano, 1999; Lawton and Edwards, 2008
7) Bateman et al., 1995; Herranz et al., 2012; Humpage et al., 2012; Khreich et al., 2010; Lawton and Edwards, 2008; Lawton et al., 2010; Meriluoto, 1997; Pelander et al., 1996; Pelander et al., 2000; Sano et al., 1992; Tong et al., 2010; Vasas et al., 2004; Xia et al., 2010; Yuan and Carmichael, 2004; Zhou et al., 2011
8) Ballot et al., 2010; Bláhová et al., 2009
9) Lawton and Edwards, 2008; Törökné et al., 2004; Welker et al., 2002
10) Ballot et al., 2010; Bláhová et al., 2009; Chen et al., 2012; Dell'Aversano et al., 2004; Eaglesham et al., 1999; Kikuchi et al., 2007; Lawton and Edwards, 2008; Oehrle et al., 2010
11) Vasas et al., 2004
12) Lawton and Edwards, 2008; Zotou et al., 1993
13) James et al., 1997; James et al., 1998; Rawn et al., 2005
14) Ballot et al., 2010; Bogialli et al., 2006; Chen et al., 2012; Dell'Aversano et al., 2004; Dimitrakopoulos et al., 2010; Furey et al., 2003; James et al., 2005; Oehrle et al., 2010
15) Zotou et al., 1993
16) Aráoz et al., 2008a; Aráoz et al., 2008b; Aráoz et al., 2010b; Azevedo et al., 2011; Vasas et al., 2004
17) Devic et al., 2002; Lawton and Edwards, 2008; Villatte et al., 2002
18) Dörr et al., 2010
19) Ballot et al., 2010; Campbell et al., 2009; Lawton and Edwards, 2008
20) Lawrence and Niedzwiadek, 2001; Lawrence et al., 2004; Lawrence et al., 2005
21) Ballot et al., 2010; Blay et al., 2011; Dell'Aversano et al., 2004; Dell'Aversano et al., 2005; Fang et al., 2004; Halme et al., 2012 ; Herrmann et al., 2012; Humpage et al., 2010; Lajeunesse et al., 2012; Lawton and Edwards, 2008; Turner et al., 2011
22) Ben-Gigirey et al., 2012 ; Campbell et al., 2009; dos Anjos et al., 2006; DeGrasse et al., 2011; Ferrão-Filho et al., 2009; Ferrão-Filho et al., 2010; Humpage et al., 2010; Lawton and Edwards, 2008; Laycock et al., 2010; Locke and Thibault, 1994; Rodríguez et al., 2010; Turner et al., 2011; Wong et al., 2010
23) Banack et al., 2010b; Cohen, 2012
24) Cohen, 2012; Faassen et al., 2012; Lawton and Edwards, 2008
25) Banack et al., 2010b; Cohen, 2012; Faassen et al., 2009; Faassen et al., 2012; Krüger et al., 2002; Kubo et al., 2008; Li et al., 2010
26) Cohen, 2012; Lawton and Edwards, 2008; Pan et al., 1997
27) Banack et al., 2010b ; Cervantes Cianca et al., 2012; Cohen, 2012; Lawton and Edwards, 2008

Table 3: Literature survey of common water treatment processes and the related impact on cyanobacteria and their toxins.

	Preoxidation	Clarification	Membrane filtration	Adsorption	Photo(cata)lysis	Ozonation	Chlorination
Cyanobacteria	*Cell lysis*[1] *Toxin release*[1]	*Cell removal*[2] *No toxin release in water*[3] *Toxin release in sludge*[4]	*Cell removal*[5] *Low cell lysis*[5]	*N.A.*[*]	*N.A.*[*]	*N.A.*[*]	*N.A.*[*]
MC	*Potential toxin alteration*[**]	*No effect*[6]	*Toxin removal*[7]	*Toxin removal*[8]	*Toxin alteration*[9]	*Toxin alteration*[10]	*Toxin alteration*[11]
NOD	*Potential toxin alteration*[**]	*No data*	*Toxin removal*[12]	*No data*	*Toxin alteration*[13]	*Toxin alteration*[14]	*Toxin alteration*[15]
CYL	*Potential toxin alteration*[**]	*No data*	*Toxin removal*[16]	*Toxin removal*[17]	*Toxin alteration*[18]	*Toxin alteration*[19]	*Toxin alteration*[20]
STX	*Potential toxin alteration*[**]	*No data*	*Toxin removal*[21]	*Toxin removal*[22]	*No data*	*Poor toxin alteration*[23]	*Toxin alteration*[24]
ANTX-a	*Potential toxin alteration*[**]	*No data*	*Toxin removal*[25]	*Toxin removal*[26]	*Toxin alteration*[27]	*Toxin alteration*[28]	*No effect*[29]
Other toxins	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*

1) Daly et al., 2007; Hall et al., 2000; Hoeger et al., 2002; Lam et al., 1995; Ma et al., 2012; Miao and Tao, 2009; Peterson et al., 1995; Zamyadi et al., 2010; Zamyadi et al., 2013

2) Chow et al., 1999; Drikas et al., 2001; Hall et al., 2000; Ho et al., 2012c; Lam et al., 1995; Ma et al., 2012; Pietsch et al., 2002; Sun et al., 2012; Teixeira and Rosa, 2006a; Teixeira et al., 2010; Velzeboer et al., 1995

3) Chow et al., 1998; Chow et al., 1999; Drikas et al., 2001; Hall et al., 2000; Peterson et al., 1995; Sun et al., 2012

4) Drikas et al., 2001; Ho et al., 2012c; Sun et al., 2012

5) Campinas and Rosa, 2010b; Chow et al., 1997; Gijsbertsen-Abrahamse et al., 2006

6) Drikas et al., 2001; Hall et al., 2000; Himberg et al., 1989

7) Campinas and Rosa, 2010a; Dixon et al., 2010; Dixon et al., 2011b; Gijsbertsen-Abrahamse et al., 2006; Hall et al., 2000; Lawton and Robertson, 1999; Lee and Walker, 2008; Neumann and Weckesser, 1998; Pantelić et al., 2013; Teixeira and Rosa, 2005; Teixeira and Rosa, 2006b; Teixeira and Rosa, 2006c; Westrick et al., 2010

8) Campinas and Rosa, 2006; Campinas and Rosa, 2010a; Delgado et al., 2012; Donati et al., 1994; Hall et al., 2000; Himberg et al., 1989; Ho et al., 2011; Huang et al., 2007; Lambert et al., 1996; Lawton and Robertson, 1999; Newcombe et al., 2003; Newcombe and Nicholson, 2004; Pantelić et al., 2013; Pendleton et al., 2001; Sorlini and Collivignarelli, 2011; Wang et al., 2007a; Warhurst et al., 1997; Westrick et al., 2010

9) Antoniou et al., 2008; Graham et al., 2010; He et al., 2012; Kaya and Sano, 1998; Lawton and Robertson, 1999; Lawton et al., 1999; Liu et al., 2003; Liu et al., 2009; Pantelić et al., 2013; Pelaez et al., 2010; Pelaez et al., 2012a; Pelaez et al., 2012b; Qiao et al., 2005; Robertson et al., 2012; Sharma et al., 2012; Shephard et al., 1998; Shephard et al., 2002; Tsuji et al., 1995; Yang et al., 2011

10) Al Momani et al., 2008; Al Momani and Jarrah, 2010; Brooke et al., 2006; Hall et al., 2000; Himberg et al., 1989; Hoeger et al., 2002; Lawton and Robertson, 1999; Miao and Tao, 2009; Miao et al., 2010; Newcombe et al., 2003; Newcombe and Nicholson, 2004; Onstad et al., 2007; Pantelić et al., 2013; Rodríguez et al., 2007a; Rositano et al., 1998; Rositano et al., 2001; Sharma et al., 2012; Shawwa and Smith, 2001; Westrick et al., 2010

11) Acero et al., 2005; Acero et al., 2008; Daly et al., 2007; Hall et al., 2000; Ho et al., 2006b; Huang et al., 2008; Ji et al., 2008; Kull et al., 2004; Kull et al., 2006; Lawton and Robertson, 1999; Merel et al., 2009; Merel et al., 2010a; Newcombe and Nicholson, 2004; Nicholson et al., 1994; Pantelić et al., 2013; Rodríguez et al., 2007a; Rodríguez et al., 2008; Senogles-Derham et al., 2003; Sharma et al., 2012; Shi et al., 2005; Sorlini and Collivignarelli, 2011; Triantis et al., 2012; Tsuji et al., 1997; Westrick et al., 2010; Xagoraraki et al., 2006; Zamyadi et al., 2012; Zamyadi et al., 2013; Zong et al., 2013

12) Lawton and Robertson, 1999; Vuori et al., 1997

13) Liu et al., 2005; Robertson et al., 2012; Twist and Codd, 1997

14) Rositano et al., 1998

15) Merel et al., 2010a; Nicholson et al., 1994; Pantelić et al., 2013

16) Dixon et al., 2010; Dixon et al., 2011b

17) Delgado et al., 2012; Ho et al., 2008; Ho et al., 2011; Newcombe and Nicholson, 2004; Pantelić et al., 2013; Westrick et al., 2010

18) Pelaez et al., 2012b; Robertson et al., 2012; Senogles et al., 2000a; Senogles et al., 2001

19) Newcombe and Nicholson, 2004; Onstad et al., 2007; Pantelić et al., 2013; Rodríguez et al., 2007a ; Westrick et al., 2010

20) Banker et al., 2001; Ho et al., 2008; Merel et al., 2010a; Merel et al., 2010b; Newcombe and Nicholson, 2004; Pantelić et al., 2013; Rodríguez et al., 2007a; Rodríguez et al., 2007b; Senogles et al., 2000b; Senogles-Derham et al., 2003; Westrick et al., 2010; Zamyadi et al., 2012

21) Laycock et al., 2012; Westrick et al., 2010

22) Delgado et al., 2012; Newcombe and Nicholson, 2004; Orr et al., 2004; Shi et al., 2012; Westrick et al., 2010

23) Newcombe and Nicholson, 2004; Orr et al., 2004; Rositano et al., 2001

24) Merel et al., 2010a; Newcombe and Nicholson, 2004; Nicholson et al., 2003; Pantelić et al., 2013; Senogles-Derham et al., 2003; Westrick et al., 2010; Zamyadi et al., 2012

25) Gijsbertsen-Abrahamse et al., 2006; Teixeira and Rosa, 2006b; Westrick et al., 2010

26) Hall et al., 2000; Newcombe and Nicholson, 2004

27) Afzal et al., 2010

28) Al Momani, 2007; Hall et al., 2000; Newcombe and Nicholson, 2004; Onstad et al., 2007; Pantelić et al., 2013; Rositano et al., 1998; Rositano et al., 2001; Rodríguez et al., 2007a; Westrick et al., 2010

29) Hall et al., 2000; Merel et al., 2010a; Newcombe and Nicholson, 2004; Rodríguez et al., 2007a; Rodríguez et al., 2007b; Westrick et al., 2010

* Cyanobacteria are not likely to reach these treatments since they are previously removed by clarification

** As a function of: raw water quality, the amount of oxidizing agent, the reactivity of the toxin with chlorine or ozone

Table 4: Overview of existing regulation on cyanotoxins in drinking water and recreational water (Chorus, 2012).

	MCs	NOD	CYL	ANTX-a	HomoANTX-a	ANTX-a(s)	STX
WHO	*1µg/L*	-	-	-	-	-	-
Australia	*1.3µg/L (10µg/L)*	-	*1µg/L*	-	-	-	*3µg/L*
Brazil	*1µg/L*	-	*15µg/L*	-	-	-	*3µg/L*
Canada	*1.5µg/L* (20µg/L)*	-	-	*3.7µg/L*	-	-	-
Czech Republic	*1µg/L**	-	-	-	-	-	-
France	*1µg/L (25µg/L)*	-	-	-	-	-	-
Germany	*1µg/L (10µg/L)*	-	*0.1µg/L*	-	-	-	-
Hungary	- *(20µg/L)*	-	-	-	-	-	-
Italy	- *(25µg/L)*	-	-	-	-	-	-
Netherland	*1µg/L (20µg/L)*						
New Zealand	*1µg/L (12µg/L)*	*1µg/L*	*1µg/L*	*6µg/L*	*2µg/L*	*1µg/L*	*3µg/L*
Singapore	*1µg/L**	-	-	-	-	-	-
Spain	*1µg/L*	-	-	-	-	-	-
Turkey	*1µg/L (25µg/L)*	-	-	-	-	-	-
Uruguay	*1µg/L*	-	-	-	-	-	-
South Africa	*1µg/L*	-	-	-	-	-	-
USA	-	-	-	-	-	-	-

** The value applies to MC-LR only*
( ) The value between parenthesis refer to recreational water